Propagule Banks in Bryophytes and Ferns: Dynamics, Genetic Composition and the Role of the Life History

Dissertation

Zur

Erlangung der naturwissenschaftlichen Doktorwürde

(Dr. sc. nat.)

vorgelegt der

Mathematisch-naturwissenschaftlichen Fakultät

der

Universität Zürich

von

Zsófia Hock

aus

Ungarn

Promotionskomitee:

Prof. Dr. Peter Linder

Prof. Dr. Elena Conti

Dr. Edwin Urmi

Dr. Jakob J. Schneller

Zürich 2007

Propagule banks in bryophytes and ferns: dynamics, genetic composition and the role of the life history

Zsófia Hock

In collaboration with: Dr. Jakob Schneller, Dr. Péter Szövényi, Dr. Zoltán Tóth and Dr. Edwin Urmi

„Und Luft und Tannen, Berge, Moos und Sterne,
Sie schlangen lächelnd ihren weiten Kranz;
Wie an der Insel sich das Meer, das ferne,
Brach sich an mir ihr friedlich milder Glanz.“

Gottfried Keller, Ein Tagewerk

Acknowledgements

I wish to thank all the people who contributed to the completion of my PhD thesis. First of all, I would like to thank Dr. Konstantin Dobolyi, who introduced me to botany. I am deeply grateful to my primary supervisors: Dr. Edwin Urmi, Prof. Dr. Jakob Schneller (Institute of Systematic Botany, University of Zurich, Switzerland) and Dr. Zoltán Tóth (Cryptogam Laboratory, Institute of Biology, Department of Plant Taxonomy and Ecology, Eötvös Loránd University, Hungary) for their guidance, support, constructive comments and our fruitful discussions. Prof. Dr. Peter Linder is gratefully acknowledged for making it possible to conduct the population genetic studies in the stimulating atmosphere of the Botanical Institute of the University of Zurich. Jakob Schneller's suggestions as a non-bryologist threw new light upon so many issues. I have always admired Edwin Urmi's exactitude in defining scientific problems and terms, his enormous knowledge on bryophytes and his delicate sense of humour. I am also very grateful for his critical comments on my manuscripts. In spite of constantly being very busy with teaching and organizing the life of the department, Zoltán Tóth has always found the time to solve my problems and to answer my questions. His friendship, technical and moral support was invaluable.

Many people have helped me along my studies. Although I can not list all of them here, their contribution is equally appreciated. I am especially grateful to my family: to my parents and grandparents, who have and introduced me to the wonders of nature and have encouraged and supported me all along my studies. My brother Ferenc was always ready to help me: his company and "protection" against dogs in the field, as well as his patience in solving my administrative problems in Hungary while I was in Switzerland, are warmly acknowledged. My friend Gábor Endresz probably knows my personal data better than I do, since he helped me so many times with the registration in my absence: thank you very much Gábor! Dr. István Pintér (Eötvös Loránd University, Hungary) helped me a lot in cultivation and determination of fern prothallia, and his admirable animation brightened my working days. My attention was drawn to propagule banks through the exciting papers of Dr. Heinjo During, who later had valuable comments on early versions of my manuscripts.

The patience and continuous assistance of my husband, Péter Szövényi, in field work and in the world of statistical analyses is invaluable. He has always encouraged my work and helped me through difficult moments.

Last but not least I would like to thank both Institutes for providing optimal working conditions. I enjoyed lunches, excursions and discussions with both my Swiss and Hungarian

colleagues and friends, in particular Alessia Guggisberg and Guilhem Mansion, Alex Bernhard, Andrea Mojzes, Anita Lendel, Baby, Balázs Könnyű, Beatriz Itten, Brigitte Marazzi, Burgi Liebst, Chloé Galley, Gábor Endresz and Imelda Somodi, Corinne Furrer and Frank Rutschmann, Cyril Guibert, Erzsébet Szurdoki and Péter Ódor, Evelin Pfeifer, Gabriele Salvo, Heike Hofmann, Ildikó László, Josefine Jacksch, Julien Bachelier, Marlon Machado, Merran Matthews, Massimo Mastracci, Monica Quast and Andre Simoes, Niklaus Müller, Norbert Schnyder, Rolf Rutishauser, Urs Landergott and Yannick Städler.

Part of my work was funded by the Hungarian Scientific Research Fund (OTKA-T047156).

Table of contents

Publication list

The following papers were published during my PhD studies. The first two publications represent earlier studies on bryophyte ecology, of which the experimental part has been carried out in Hungary. Publications III, IV and VI are the result of a joint research on peat mosses with Péter Szövényi. Publications V, VII, VIII, containing the results of my PhD research, are included in the thesis.

I. **Hock Zs,** Szövényi P, Tóth Z 2004 Seasonal variation in the diaspore bank of bryophytes in open dolomite rock grasslands. *Journal of Bryology* **26**: 285-292

II. Szövényi P, **Hock Zs**, Tóth Z 2004 Phorophyte preferences of epiphytic bryophytes in a stream valley in the Carpathian Basin. *Journal of Bryology* **26**: 137-146

III. Szövényi P, **Hock Zs**, Urmi E, Schneller JJ 2006 New primers for amplifying the GapC gene of bryophytes and its utility in intraspecific phylogenies in the genus *Sphagnum*. *Lindbergia* **31**: 78-84

IV. Szövényi P, **Hock Zs**, Urmi E, Schneller JJ 2006 Contrasting phylogeographic patterns in *Sphagnum fimbriatum* and *S. squarrosum* (Bryophyta, Sphagnopsida) in Europe. *New Phytologist* **172**: 784-794

V. **Hock Zs**, Szövényi P, Tóth Z 2006 Seasonal variation in the spore bank of ferns in grasslands on dolomite rock. *Plant Ecology* **187**: 289-296

Articles in review

VI. **Szövényi P**, Hock Zs, Schneller JJ, Urmi E, Tóth Z 2006 Does multilocus dataset support cryptic demographic histories in two peat mosses in Europe? In review, *BMC Evolutionary Biology*

VII. **Hock Zs**, Szövényi P, Tóth Z 2007 Effects of habitat type and seasons on the composition of the bryophyte diaspore bank and its relation to the standing vegetation. In review: *Plant Ecology*

VIII. **Hock Zs**, Szövényi P, Schneller JJ, Urmi E, Tóth Z 2007 Population genetic consequences of the reproductive system in the liverwort *Mannia fragrans* (Aytoniaceae) In review: *American Journal of Botany*

Manuscripts in preparation

IX. **Hock Zs**, Szövényi P, Schneller JJ, Tóth Z 2007 Bryophyte diaspore bank – a genetic memory? Genetic structure and genetic diversity of surface populations and diaspore bank in the liverwort *Mannia fragrans* (Balb.) Frye and Clark

General introduction

The expression „diaspore bank“ covers the totality of viable sexual and asexual propagules on or within the substrate. While bryophyte diaspore banks are composed of both types of propagules (During 2001), fern diaspore banks almost exclusively contain spores (During and ter Horst 1983). Therefore the terms “diaspore bank” and “spore bank” will be used to distinguish between the propagule banks of the two plant groups.

Compared with the seed bank of vascular plants, relatively few studies have investigated the diaspore banks of bryophytes and ferns. Published work focuses on various aspects of the diaspore bank including its composition in different habitats, at different depths, and on its relationship to the above-ground vegetation at a given time (c.f. During 2001 for a review on bryophytes; Leck and Simpson 1987, Dyer and Lindsay 1992, Ramírez-Trejo et al. 2004). However, most studies provide a static image, and seasonal dynamics have received little attention so far (During and ter Horst 1983, During et al. 1987). The number of successful attempts to relate changes in the diaspore bank to those in the standing vegetation is also low (During et al. 1987, During and Lloret 1996, Ghorbani et al. 2003).

The role of diaspore banks is multifarious: they allow the species to bridge unfavourable periods, to recolonize after disturbance, and they may conserve genetic variability within populations (During 2001). Mathematical and theoretical models (e.g. Templeton and Levin 1979, Vitalis et al 2004) have generated several hypotheses about the evolutionary consequences of propagule banks, including consequences on the genetic structure of populations. According to these models, propagule banks may act as reservoirs for genetic diversity, buffering population genetic consequences of bottlenecks (del Castillo 1994). They can also function as a “genetic memory” and store genotypes from several generations formed under potentially different selective pressures (Cabin et al. 1998). These predictions are now supported by an increasing amount of empirical data in vascular plants (Bennington et al. 1991, Tonsor et al 1993, Cabin 1996, Alvarez-Buylla et al. 1996, McCue and Holtsford 1998, Mahy et al 1999, Nunney et al. 2002, Koch et al. 2003, Barrett et al. 2005, Shimono et al. 2006). However, patterns detected in these studies differ among species and are probably largely dependent on life history traits and habitat characteristics. It is thus still difficult to draw general trends. In bryophytes, the diaspore bank was postulated to be able to conserve genetic variability and thus influence genetic structure of extant populations (During 1997). However, to date no studies have attempted to assess this hypothesis experimentally. Since propagule banks may help to understand dynamics of populations, patterns of variation in a

historical context and to restore genetic variation lost from populations at the surface (McGraw 1993, Morris et al. 2002, Uehara et al. 2006), such investigations could be of great importance in understanding the population biology of bryophytes.

To interpret the composition and dynamics of the diaspore bank, knowledge on the life cycle and behaviour of the species forming it is necessary. Bryophytes have been grouped into life history strategy categories by several authors (e.g. During 1979, 1992, Frey and Kürschner 1991). These classifications provide an important tool for recognizing relationships between environment and life cycle characteristics. They are widely used in practice to characterize communities and to estimate the survival ability and evolutionary capacity of individual species (Papp et al. 2005, Hallingbäck et al. 1998). During (1979) was the first to establish distinct categories based on two major ecological trade-offs: spore size versus spore number and allocation between reproduction and longevity. However, as some categories proved to be too heterogeneous, he later divided them into subcategories (During 1992). Frey & Kürschner (1991) proposed a slightly different, detailed classification based on reproductive characteristics. These classifications all contain hypothetical elements and thus assignment of species to a limited number of discrete categories may be problematic due to strategy overlap in nature (Longton 1997). More or less intuitive lists assigning species to life history strategies have been compiled by several authors (e.g. Orbán 1983; Dierßen 2001), but recent experiments show that they often need revision. Empirical data about the major elements of the life cycle of individual species and their interplay have been accumulated over the last few years and a database is being compiled merging old literature and recent field data (Söderström and Gunnarsson 2003). However, some elements such as reproductive system, sex expression rates, distribution of sexes in a unisexual species, interplay between asexual and sexual reproduction and costs of reproduction remain relatively unexplored (cf. Stark 2002 for a review).

The reproductive system in bryophytes includes almost every possible combination of sexual organs (Wyatt and Anderson 1984). In the two main cases, male and female gametangia may appear either on separate individuals (unisexual) or on the same gametophyte (bisexual). To avoid ambiguity, the terms unisexual and bisexual will consequently be applied within this thesis to denote these two cases. Although it may seem simple to determine whether a species is uni- or bisexual, practice shows that it is not always the case. Separate individuals of seemingly unisexual species may turn out to have germinated from the same spore and to be connected by rhizoids or protonema underground (Stark and Delgadillo 2001) and, with increasing number of empirical studies, several other pitfalls emerge. In spite of the

early discovery of the existence of sex chromosomes (Allen 1930), sex is not always genetically determined: it may depend on the age of the plants (Wyatt and Anderson 1984) and/or environment may also considerably influence it (Korpelainen 1998). A relatively rare and intriguing reproductive strategy is polyoecy. There exist several definitions for this term and it is not always clear whether it refers to an individual or to a group of plants. In the thesis it will be used in the sense "trioecious" (Wyatt 1985), designating plant populations consisting of bisexual gametophores and unisexual gametophores of both sexes. Polyoecy has been suggested in 7.4% of all moss species (Wyatt and Anderson 1984) and about 5% of all liverwort species (based on Paton 1999) occurring in the British Isles. Apart from statements about polyoecy related to taxonomic questions and identification of species (Wyatt and Anderson 1984, Paton 1999), there are no data available about the functioning of this reproductive system, or the reasons, dynamics, frequency and advantages of co-occurrence of uni- and bisexual plants.

Biased sex ratios at the ramet level seem to be common in bryophytes, with the prevalence of females in most cases (Bisang and Hedenäs 2005) but little is known about sex ratios at the genet level due to the lack of appropriate sex-specific markers. Causes underlying this phenomenon may be multifarious but are to date poorly understood. Among others, they include genetic control, differential tolerance, survival and clonal growth rates of the two sexes (Bisang and Hedenäs 2005). Differential cost of realized reproduction was also expected to contribute to these patterns (Stark et al. 2000, Crowley et al. 2005). Higher average cost of sexual reproduction in males was proposed to be at the origin of female biased sex ratios (Stark et al. 2000), however, according to recent studies, its role is questionable in many cases (Bisang and Hedenäs 2005, Bisang et al. 2006). Moreover, although estimation of the cost of reproduction in bryophytes was postulated to be less biased by differential resource acquisition than in vascular plants, due to their low ability to compensate for it (lack of below-ground structures specialized for storage - Rydgren and Økland 2003), it is still relatively easy to erroneously interpret patterns detected. Cost of reproduction in different sexes may be estimated comparing gametangia masses, or it may be assessed as a trade-off between the production of gametangia and future performance (Bisang and Hedenäs 2005), the latter method being only rarely applied (Stark 2002). In case of limited resources, a fertilized female plant, responsible for the formation of sporophytes, should invest less energy into growth than males (Proctor 1984) or unfertilized females (Bisang and Ehrlén 2002).

Sexual reproduction in bryophytes is completed by various ways of asexual propagation, including clonal propagation by fragmentation and production of specialized asexual

propagules (Longton and Schuster 1983). In this study, fertilization between identical clones and intra-gametophytic selfing are considered to be analogous to asexual reproduction, since they yield completely homozygous spores that are postulated to be functionally comparable to asexual propagules (Wyatt et al. 1989). A competition for limited resources may exist between the sporophyte and the gametophyte (Bisang and Ehrlén 2002, Laaka-Lindberg 2001) resulting in an antagonism between sexual and asexual reproduction. However, temporal separation of the these two methods of reproduction can be beneficial to the populations: asexual reproduction, which is believed to require less energy than sexual reproduction, may prove crucial under suboptimal (e.g. stress, absence of partner) conditions (Longton and Schuster 1983, Newton and Mishler 1994) such as in unpredictable, temporal or extreme habitats (During 1992). In addition, the two ways of reproduction often play different roles in the dynamics of populations (Newton and Mishler 1994, Green and Noakes 1995). Investigation of the genetic structure within and among populations of individual species may help to clarify the relative importance of sexual and asexual reproduction (Van Der Velde et al. 2001a,b).

Detailed studies on individual species combining ecological and molecular tools are likely to bring a significant advance in the field of reproductive ecology (Longton 1994, During 2006). Life history traits may considerably influence genetic structure within and among populations causing the non-random distribution of genotypes (Hamrick and Godt 1996). In particular, ecological factors influencing reproduction and dispersal may be especially important in the development of the genetic structure (Loveless and Hamrick 1984, Wyatt 1994). Uneven sex ratios, or the complete absence of one or both sexes may limit or hamper fertilization success (McLetchie 1992, Bisang et al. 2004) and result in impoverishment in genetic variation. Differences in dispersal potential also have a severe impact on the distribution of gene diversity. Although colonization events may periodically reduce allelic diversity, species with high dispersal potential and frequent sexual reproduction are usually supposed to have high gene diversity throughout their distributional range due to admixture. Contrarily, species relying on short-range dispersal only may go through severe bottlenecks and experience high genetic drift. If no intermixture with other populations takes place, populations of these species may show decreased allelic richness and reduced gene diversity.

Our studies were conducted in Hungarian grasslands, mostly on dolomite bedrock. Dolomite hills occur in large areas of the Pannonian Basin. Grasslands developing on such hills have very early attracted the attention of botanists and ecologists alike, because of their

diversity and richness in endemic and relic species (Zólyomi 1942, 1987, Draskovits 1967). These habitats are also special because of the interplay between the physico-chemical properties of the bedrock and difference in exposure, which give rise to adjacent vegetation types with essentially different microclimate, structure and species composition (Draskovits and Kovács-Láng 1968, Bartha et al. 1998). North-facing slopes are occupied by species-rich, closed grasslands composed of perennials and tall grasses, due to their more constant climatic conditions. On southern exposed sites, steep slopes, severe abiotic stress (great daily variations in soil temperature and moisture conditions) and low temporal predictability of abiotic conditions result in the establishment of xerothermic open grasslands with sparse vegetation dominated by ephemerals, annuals and short turf fescues. In spite of the great interest of botanists and the large number of ecological investigations on vascular plants and seed bank of these sites (Bartha et al. 1998; Csontos et al. 1996; 2004), bryological studies are limited to floristic inventories alone (Dobolyi et al. 1991).

An intriguing species which is known to occur in the open grasslands is the scented liverwort, *Mannia fragrans* (Balb.) Frye and Clark, the model species of our population genetic studies. *Mannia fragrans* is one of the relatively rare xero- and thermophytic liverwort species: it occurs in more or less isolated stands of open grasslands. Being a short-lived shuttle strategist (sensu During 1979, Vojtkó 1998), it mainly appears on the soil surface in spring and in autumn, when temperature and moisture conditions are most favourable. Thalli die or become inconspicuous in unfavourable periods. It has developed several protective traits and mechanisms against drought, including light-reflecting ventral scales, thick-walled cells in the dorsal epidermis and slight sinking of the enrolled thalli into the soil during dry periods (Damsholt 2002).

Mannia fragrans has an interesting reproductive system: it is described as polyoicous, but bisexual plants seem to be generally rare if at all present (Schuster 1992, Damsholt 2002, and pers. obs.). Schuster (1992) suggested that the species is most likely be genetically bisexual, however in different periods or in different populations only unisexual plants are present. Both sexual reproduction by spores and asexual propagation by fragmentation are frequent (Damsholt 2002), resulting in dense patches of intermingled thalli of about 10 cm diameter. In spite of the abundant spore production in spring, probably only a very small fraction of the large spores (60-80μm, Damsholt 2002) is able to reach remote sites due to negative correlation between spore size and effectiveness of long-range dispersal (van Zanten 1978, van Zanten and Gradstein 1988, Miles and Longton 1992, Söderström and Herben 1997). However, spores may remain viable for several years in the diaspore bank (2-3 years,

pers. obs.). Larger amounts of viable spores of bryophytes may be stored in the soil for longer periods, even when the gametophytes disappear from the surface (During 1997, 2001).

Earlier studies on population genetics of *M. fragrans* using allozymes (Szweykowski and Odrzykoski 1981) reported low polymorphism in northern populations of the species, but found polymorphism in Hungarian populations. As allozymes provide relatively low resolution, genotypes which proved to be identical using allozyme methods often turn out to be genetically different when using high resolution DNA-fingerprinting methods (Cronberg 2002). DNA-based molecular methods are nowadays successfully used to answer different ecological questions (e.g. Van der Velde et al. 2001a,b, Snäll et al. 2004). ISSR (Inter Simple Sequence Repeat, Zietkiewicz et al. 1994, Gupta et al. 1994) markers have recently been used to study small scale genetic variation in bryophyte populations (Hassel and Gunnarson 2003, Vanderpoorten et al. 2003). Published studies (cf. Wolfe and Liston 1998 for a rewiev) emphasize the high reproducibility and fidelity of this method, which is due to longer primer sequences and high annealing temperatures enabling specific annealing of primers. A further advantage of the method is that it only requires small template DNA quantities (5-10 ng per reaction). Based on these properties, and the relative cost effectiveness of the method, ISSR markers were employed to study genetic variation in populations of *M. fragrans*.

This thesis consists of four chapters. In the first chapter, I explored the composition of the bryophyte diaspore bank in neighbouring open and closed grasslands on dolomite rock by means of relevés and cultivated soil samples taken seasonally from the intersections of a permanent grid. I aimed to detect any effects of the habitat type (openness) and seasonal changes in environmental parameters on the composition of the diaspore bank and to relate these changes to changes in the standing vegetation. As life history strategies of individual species may help to understand dynamics above-ground and in the diaspore bank (During 1997, 2001), particular attention was paid to them in the interpretation of the results. Unexpectedly, a considerable number of fern prothallia emerged from the samples, which encouraged me to additionally study the patterns and dynamics of these plants and to compare them with bryophyte results.

In the second chapter, I tested whether similar seasonal variations may occur in the spore bank of ferns from the same study sites. Such variations may either be related to spore dispersal periods of the species (During and ter Horst 1983) or to seasonally varying environmental conditions. I additionally tested the viability of spores by means of storing parts of the soil samples for periods of 6-12 months. I was aware that this method provides information on the viability of spores in a desiccated state neglecting many factors acting in

natural conditions. However, in situ burial of soil samples was not possible because of the negative experience with tourists destroying markings in the field.

The third chapter investigates the life history of *Mannia fragrans*, a frequent component of the bryophyte flora and diaspore bank in open grasslands. Special interest was paid to the reproductive ecology of the species and to its repercussions on the genetic structure of populations. Factors influencing sex expression, contributing to the development of biased sex ratios and maintaining the polyoicous reproductive system are discussed. ISSR markers and cultivation experiments helped to determine the relative importance of clonal propagation and sexual reproduction by spores.

The fourth chapter aims to test whether the diaspore bank of bryophytes may function as a reservoir of genetic diversity and variability using *Mannia fragrans* as a model system. This species has been selected on the basis of its large, probably longer lived spores forming a large diaspore bank (Hock 2003), life strategy, preference towards habitats with shorter life-span, and genetically polymorphic populations. These traits predicted that the diaspore bank of the species may play a role in conserving genetic diversity. ISSR markers were used to detect differences in the genetic composition between (1) populations above ground and in the diaspore bank (2) samples from different seasons.

The lack of experimental data on the reproductive ecology and life history of bryophyte species represent the starting point for the present work. The four chapters of the thesis investigate different aspects of these questions, from the community level to individual species, and attempt to determine directions for future bryological studies.

References

Allen CE 1930 Inheritance in a hepatic. *Science* **71**: 197-204

Alvarez-Buylla ER, Chaos Á, Piñero D, Garay AA 1996 Demographic genetics of a pioneer tropical tree species: patch dynamics, seed dispersal, and seed banks. *Evolution* **50**: 1155-1166

Bartha S, Rédei T, Szollát Gy, Bódis J, Mucina L 1998 Északi és déli kitettségű dolomitsziklagyepek térbeli mintázatainak összehasonlítása. In: Csontos P (ed) *Sziklagyepek szünbotanikai kutatása.* Scientia Kiadó, Budapest

Barrett LG, He T, Lamont BB, Krauss SL 2005 Temporal patterns of genetic variation across a 9-year-old aerial seed bank of the shrub *Banksia hookeriana* (Proteaceae). *Mol Ecol* **14**: 4169-4179

Bennington CC, McGraw JB, Vavrek MC 1991 Ecological genetic variation in seed banks. II. Phenotypic and genetic differences between young and old subpopulations of *Luzula parviflora*. *J Ecol* **79**: 627-643

Bisang I, Ehrlén J 2002 Reproductive effort and cost of sexual reproduction in female *Dicranum polysetum*. *Bryologist* **105**: 384-397

Bisang I, Ehrlén J, Hedenäs L 2004 Mate limited reproductive success in two dioicous mosses. *Oikos* **104**: 291-298

Bisang I, Hedenäs L 2005 Sex ratio patterns in dioicous bryophytes re-visited. *J Bryol* **27**: 207-219

Bisang I, Ehrlén J, Hedenäs L 2006 Reproductive effort and costs of reproduction do not explain female-biased sex ratios in the moss *Pseudocalliergon trifarium* (Amblystegiaceae). *Am J Bot* **93**:1313-1319

Cabin RJ 1996 Genetic comparison of seed bank and seedling population of the perennial desert mustard, *Lesquerella fendleri*. *Evolution* **50**: 1830-1841

Cabin RJ, Mitchell RJ, Marshall DL 1998 Do surface plant and soil seed bank populations differ genetically? A multipopulation study of the desert mustard *Lesquerella fendleri* (Brassicaceae). *Am J Bot* **85**: 1098-1109

Cronberg N 2002 Colonization dynamics of the clonal moss *Hylocomium splendens* on islands in a Baltic land uplift area: reproduction, genet distribution and genetic variation. *J Ecol* **90**: 925-935

Crowley PH, Stieha CR, McLetchie DN 2005 Overgrowth competition, fragmentation and sex-ratio dynamics: a spatially explicit, sub-individual-based model. *J Theor Biol* **233**: 25-42

Csontos P, Tamás J, Kalapos T 1996 Soil seed banks and vegetation recovery on dolomite hills in Hungary. *Acta Bot Hung* **40**: 35-43

Csontos P, Tamás J, Podani J 2004 Slope aspect affects the seed mass spectrum of grassland vegetation. *Seed Sci Res* **14**: 379-385

Damsholt K 2002 *Illustrated flora of Nordic liverworts and hornworts.* Nordic Bryological Society, Lund, Sweden

del Castillo RF 1994 Factors influencing the genetic structure of *Phacelia dubia*, a species with a seed bank and large fluctuation in population size. *Heredity* **72**: 446-458

Dierßen K 2001 Distribution, ecological amplitude and phytosociological characterization of European bryophytes. *Bryophytorum Bibliotheca* **56**

Dobolyi K, Kováts D, Szerdahelyi T, Szollát Gy 1991 Vegetation studies on the rocky grasslands of Odvas Hill (Budaörs, Hungary). *Ann Hist-Nat Mus Nat Hung* **83**: 199-223

Draskovits R 1967 A *Linum dolomiticum* Borb. cönológiai viszonyai. *Bot Közl* **54**: 193-201

Draskovits R, Kováncs-Láng E 1968 Mikroklimamessungen in Kalkstein- und Dolomitfelsenrasen. *Ann Univ Sci Budapestinensis, Sect Biol* **9-10**: 115-129

During HJ 1979 Life strategies of bryophytes: a preliminary review. *Lindbergia* **5**: 2-18

During HJ 1992 Ecological classifications of bryophytes and lichens In: Bates JW, Farmer AM (eds) *Bryophytes and lichens in a changing environment.* pp 1-31. Oxford University Press, Oxford

During HJ 1997 Bryophyte diaspore banks. *Adv Bryol* **6**: 103-134

During HJ 2001 Diaspore banks. *Bryologist* **104**: 92-97

During HJ 2006 Trends in bryophyte population dynamics. *Lindbergia* **1-2**: 6-16

During HJ, Bruguès M, Cros RM, Lloret F 1987 The diaspore bank of bryophytes and ferns in the soil in some contrasting habitats around Barcelona, Spain. *Lindbergia* **13**: 137-149

During HJ, Lloret F 1996 Permanent grid studies in bryophyte communities. I. Pattern and dynamics of individual species. *J Hattori Bot Lab* **79**: 1-41

During HJ, ter Horst B 1983 The diaspore bank of bryophytes and ferns in chalk grassland. *Lindbergia* **9**: 57-64

Dyer AF, Lindsay S 1992 Soil spore banks of temperate ferns. *Am Fern J* **82**: 89-123

Fekete G, Kovács M 1978 The space dynamism of species and life form diversity in two rocky grassland communities. *Acta Biol Debr* **15**: 7-21

FreyW, Kürschner H 1991 Das *Fossombronio-Gigaspermetum mouretii* in der Judäischen Wüste. 2. Ökosoziologie und Lebensstrategien. *Crypt Bot* **2**: 73-84

Ghorbani J, Das PM, Das AB, Hughes JM, McAllister HA, Pallai SK, Pakeman RJ, Marrs RH, Le Duc MG 2003 Effects of restoration treatments on the diaspore bank under dense *Pteridium* stands in the UK. *Appl Veg Sci* **6**: 189–198

Green RF. Noakes DLG 1995 Is a little bit of sex as good as a lot? *J Theor Biol* **174**: 87-96
Newton AE, Mishler BD 1994 The evolutionary significance of asexual reproduction in mosses. *J Hattori Bot Lab* **76**: 127-145

Gupta M, Chyi Y-S, Romero-Severson J, Owen JL 1994 Amplification of DNA markers from evolutionarily diverse genomes using single primers of simple-sequence repeats. *Theor App Gen* **89**: 998-1006

Hallingbäck T, Hodgetts NG, Raeymakers G, Schumacker R, Sérgio C, Söderström L, Stewart N, Váňa J 1998 Guidelines for application of the revised IUCN threat categories to bryophytes. *Lindbergia* **23**: 6–12

Hamrick JL, Godt MJW 1996 Effects of life history traits on genetic diversity in plant species. *Phil Trans R Soc Lond B* **351**: 1291-1298

Hassel K, Gunnarson U 2003 The use of inter simple sequence repeats (ISSR) in bryophyte population studies. *Lindbergia* **28**: 152-157

Hock Zs 2003 *A kriptogám diasprórabank vizsgálata dolomit sziklagyepekben.* [The diaspore bank of cryptogams in grasslands on dolomite rock.] MSc thesis, Eötvös Loránd University, Department of Plant Taxonomy and Ecology, Budapest, Hungary

Koch M, Huthmann M, K-G Bernhardt 2003 *Cardamine amara* L. (Brassicaceae) in dynamic habitats: Genetic composition and diversity of seed bank and established populations. *Basic Appl Ecol* **4**: 339-348

Korpelainen H 1998 Labile sex expression in plants. *Biol Rev* **73**: 157-180

Laaka-Lindberg S 2001 Biomass allocation to sexual and asexual reproduction in a leafy hepatic *Lophozia silvicola* Buch. *J Bryol* **23**: 3-8

Leck MA, Simpson RL 1987 Spore bank of a Delaware River freshwater tidal wetland. *Bull Torrey Bot Club* **114**: 1-7

Longton RE 1994 Reproductive biology in bryophytes: the challenge and the opportunities. *J Hattori Bot Lab* **75**: 1-13

Longton RE 1997 Life-history strategies, an intuitive approach. *Adv Bryol* **6**: 66-101

Longton RE, Schuster RM 1983 Reproductive biology. In: Schuster RM (ed) *New manual of bryology.* Vol 1. pp 386-462. Hattori Bot Lab, Nichinan

Loveless MD, Hamrick JL 1984 Ecological determinants of genetic structure in plant populations. *Ann Rev Ecol Syst* 15: 65-95

Mahy G, Vekemans X, Jacquemart A 1999 Patterns of allozymic variation within *Calluna vulgaris* populations at seed bank and adult stages. *Heredity* **82**: 432-440

McCue KA, Holtsford TP 1998 Seed bank influences genetic on genetic diversity in the rare annual *Clarkia springvillensis* (Onagraceae). *Am J Bot* **85**: 30-36

McGraw JB 1993 Ecological Genetic Variation in Seed Banks. IV. Differentiation of Extant and Seed Bank-Derived Populations of *Eriophorum vaginatum*. *Arct Alp Res* 25: 45-49

McLetchie DN 1992 Sex ratio from germination through maturity and its reproductive consequences in the liverwort *Sphaerocarpos texanus*. *Oecologia* **92**: 273-278

Miles CJ, Longton RE 1992 Deposition of moss spores in relation to distance from parent gametophytes. *J Bryol* **17**: 355–368

Morris AB, Baucom RS, Cruzan MB 2002 Stratified analysis of the soil seed bank in the cedar glade endemic *Astragalus bibullatus*: evidence for historical changes in genetic structure. *Am J Bot* **89**: 29-36

Newton AE, Mishler BD 1994 The evolutionary significance of asexual reproduction in mosses. *J Hattori Bot Lab* **76**: 127-145

Nunney L 2002 Effective size of annual plant populations: the interaction of a seed bank with fluctuating population size in maintaining genetic variation. *Am Nat* **160**: 195-204

Orbán S 1983 *A magyarországi mohák stratégiái, összefüggésük a környezet ökológiai és cönológiai jellemzőivel.* PhD dissertation, Eötvös Loránd University, Budapest, Hungary

Papp B, Ódor P, Szurdoki E 2005 Methodological overview and a case study of the Hungarian bryophyte monitoring program. *Bol Soc Esp Briol* **26-27**: 23-32

Paton JA 1999 *The liverwort flora of the British Isles.* Harley Books, UK

Proctor MCF 1984 Structure and ecological adaptation. In: Dyer AF and Duckett JG (eds) *The experimental biology of bryophytes.* pp 9–37. Academic Press, London, UK

Ramírez-Trejo MdR, Pérez-Garcia B, Orozco-Segovia A 2004 Analysis of fern spore banks from the soil of three vegetation types in the central region of Mexico. *Am J Bot* **9**: 862-688

Rydgren K, Økland RH 2003 Short-term costs of sexual reproduction in the clonal moss *Hylocomium splendens*. *Bryologist* **106**: 212-220

Schuster RM 1992 *The Hepaticae and Anthocerotae of North America Vol. VI.* Field Museum of Natural History, Chicago

Shimono A, Ueno S, Tsumura Y, Washitani I 2006 Spatial genetic structure links between soil seed banks and above-ground populations of *Primula modesta* in subalpine grassland. *J Ecol* **94**: 77-86

Snäll T, Fogelqvist J, Ribeiro PJ, Lascoux M 2004 Spatial genetic structure in two congeneric epiphytes with different dispersal strategies analysed by three different methods. *Mol Ecol* **13**: 2109-2119

Söderström L, Gunnarsson U (compilers) 2003 *Life history strategies. A catalogue of population biology parameters for bryophytes occurring in North-Western Europe.* Manual v. 1.0, BryoPlanet, Trondheim. http://www.bio.ntnu.no/users/gunnarss/Hemsida [accessed on 21.02.2007]

Söderström L, Herben T 1997 Dynamics of bryophyte metapopulations. *Adv Bryol* **6**: 205-240

Stark LR 2002 Phenology and its Repercussions on the Reproductive Ecology of Mosses. *Bryologist* **105**: 204-218

Stark LR, Mishler BD, McLetchie DN 2000 The cost of realized sexual reproduction: assessing patterns of reproductive allocation and spore abortion in a desert moss. *Am J Bot* **87**: 1599-1608.

Stark LR, Delgadillo MC 2001 Rhizoautoicous *Aloina bifrons* in the Mojave Desert, a possible adaptation to increase spore production. *Bryologist* **104**: 104-108

Templeton AR, Levin DA 1979 Evolutionary consequences of seed pools. *Am Nat* **114**: 232-249

Tonsor SJ, Kalisz S, Fisher J, Holtsford TP 1993 A life-history based study of population genetic structure: seed banks to adults in *Plantago lanceolata*. *Evolution* **47**: 833-843

Uehara K, Tanaka N, Momohara A, Zhou Z-K 2006 Genetic diversity of an endangered aquatic plant, *Potamogeton lucens* subspecies *sinicus*. *Aqu Bot* **85**: 350-354

Vanderpoorten A, Hedenäs L, Jacquemart AL 2003 Differentiation in fingerprinting and morphology among species of the pleurocarpous moss genus, *Rhytidiadelphus* (Hylocomiaceae). *Taxon* **52**: 229-236

Van der Velde M, During HJ, Van de Zande L, Bijlsma R 2001a The reproductive biology of *Polytrichum formosum*: clonal structure and paternity revealed by microsatellites. *Mol Ecol* **10**: 2423-2434

Van Der Velde M, Van De Zande L, Bijlsma R 2001b Genetic structure of *Polytrichum formosum* in relation to the breeding system as revealed by microsatellites. *J Evol Biol* **14**: 288-295

Van Zanten BO 1978 Experimental studies on trans-oceanic long-range dispersal of moss spores in the southern hemisphere. *J Hattori Bot Lab* **44**: 455–482

Van Zanten BO, Gradstein SR 1988 Experimental dispersal geography of neotropical liverworts. *Beiheft Nova Hedw* **90**: 41–94

Vitalis R, Glémin S, Olivieri I 2004 When genes go to sleep: The population genetic consequences of seed dormancy and monocarpic perenniality. *Am Nat* **163**: 295–311

Vojtkó A 1998 Relationships between the morphology of the spores of Hungarian Hepaticae species and their strategy types. *Studia Bot Hung* **27-28**: 109-115

Wolfe AD, Liston A 1998 Contributions of PCR-based methods to plant systematics and evolutionary biology. In: Soltis DE, Soltis PS, Doyle JJ (eds) *Plant Molecular Systematics II.* pp 43-86. Kluwer

Wyatt R 1985 Terminology for bryophyte sexuality: toward a unified system. *Taxon* **34**: 420-425

Wyatt R 1994 Population genetics of bryophytes in relation to their reproductive biology. *J Hattori Bot Lab* **76**: 147-157

Wyatt R, Anderson LE 1984 Breeding systems in bryophytes. In Dyer AF, Duckett JG (eds) *The experimental biology of bryophytes.* pp 39-46. Academic Press, Orlando, FL.

Wyatt R, Stoneburner A, Odrzykoski I 1989 Bryophyte isozymes: Systematic and evolutionary implications. In: Soltis DE, Soltis PS (eds) *Plant Isozymes.* Dioscorides Press, Portland, OR

Zietkiewicz E, Rafalski A, Labuda D 1994 Genome fingerprinting by simple sequence repeat (SSR)-anchored polymerase chain reaction amplification. *Genomics* **20**: 176-183

Zólyomi B 1942 A középdunai flóraválasztó és a dolomitjelenség. Die Mitteldonau-Florenscheide und das Dolomitphänomen. *Bot Közl* **39**: 209–231

Zólyomi B 1987 Coenotone, ecotone and their role in preserving relic species. *Acta Bot Hung* **33**: 3–18

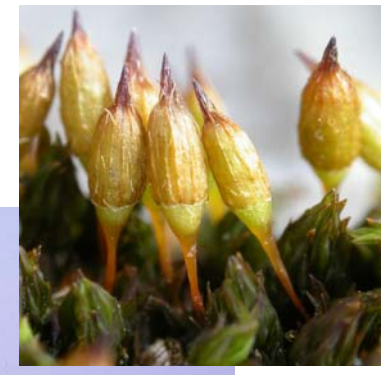
Chapter I.

Effects of habitat type and seasons on the composition of the bryophyte diaspore bank and its relation to the standing vegetation

Zsófia Hock[1,2], Péter Szövényi[1,2] and Zoltán Tóth[2]

[1]*Institute of Systematic Botany, University of Zürich, Zürich, 8008, Zollikerstr. 107, Switzerland;*
[2]*Eötvös Loránd University, Budapest, 1117, Pázmány Péter s. 1/C, Hungary*

Under review in
Plant Ecology



Abstract

Adjacent grasslands on dolomite rock with different exposure house fundamentally different plant communities due to the physico-chemical properties of the bedrock. The effect of grassland type on the composition of the bryophyte vegetation was tested and changes in the bryophyte diaspore bank and in the standing vegetation related to seasonally varying factors were connected. Specifically, the following questions were tested: Is species composition and cover of bryophytes above and below ground correlated with openness of grasslands? Do relationships between above- and below-ground patterns change seasonally? And finally, do changes correlate with seasonal variations of environmental factors and presumed life strategies of species? Total cover of bryophytes was estimated in relevés and soil samples taken seasonally using a permanent grid in two open and a closed grasslands on dolomite rock in Hungary. Frequencies of species were also established. Species composition and life strategy patterns differed according to habitat type, with colonists and shuttle strategist dominating in open grasslands, and perennials in closed grasslands. Cover of bryophytes at the surface was higher in closed grasslands but this relationship reversed in the diaspore bank. In arid open grasslands, the dominating highly reproductive species formed large diaspore banks, but only appeared above ground periodically. In closed grasslands with more constant environmental conditions, the proportion of perennials with lower reproductive effort was higher. Cover of bryophytes decreased from August 2001 to June 2002 above ground but increased in cultivated soil samples. Unfavourable seasons caused the recession of short-lived species to the diaspore bank. Advantages of parallel analysis of the standing vegetation and the diaspore bank may help to clarify the dynamics of individual species and the whole bryophyte community.

Keywords: life strategies; spore bank; vegetation type

Introduction

Grasslands on dolomite rock have always been an attractive subject for ecological investigations due to their exceptional ecological properties (Draskovits and Kovács-Láng 1968). Interactions between the physico-chemical features of the bedrock and exposure result in establishment of proximate but fundamentally different plant communities (Bartha et al. 1998; Draskovits and Kovács-Láng 1968). South-facing slopes house open grasslands, with shallow, moving, rendzina soil and sparse patches of vegetation dominated by ephemerals, annuals and short, tussocky fescues. North-facing slopes house closed grasslands with perennial species and dense turfs of tall grasses allowing the accumulation of a deeper humus layer. The microclimate of the two habitats differs clearly: arid in open grasslands the microclimate is arid, with large daily and seasonal temperature fluctuations, while in closed grasslands moisture and temperature conditions are more even constant (Csontos et al. 2004; Draskovits and Kovács-Láng 1968). However, both vegetation types share similar main growth periods, in spring and autumn; alternating with less favourable conditions in summer and winter. Although, comparative works on ecology, dynamics and seed banks of vascular plants in the two grassland types are numerous (Bartha et al. 1998; Csontos et al. 1996; 2004; Draskovits and Kovács-Láng 1968), bryological studies have to date, only focused on the floristic aspects of these sites.

In order to gain an understanding of the bryophyte vegetation of these grasslands, a parallel analysis of the diaspore bank is crucial, since many of the species present only appear at the surface periodically (During 1992). Seed bank and overlaying vegetation are often coupled: their composition and abundance may be significantly correlated (Arroyo et al. 1999; Grillas et al. 1993; Henderson et al. 1988). Since the diaspore bank of bryophytes is usually persistent, and hence capable of conserving viable propagules for several years (During 1997; 2001), similar patterns are hypothesized for the bryophyte diaspore bank as well. However, successful experimental approaches which test this are sparse (During et al. 1987; During and Lloret 1996, Ghorbani et al. 2003). The number of propagules stored in the diaspore bank is not constant, and varies considerably depending on seasons and life-history characteristics, which in turn, may be coupled with changes in the standing vegetation (Hock et al. 2004).

Therefore the aim of this investigation was to elucidate, whether species composition and cover of bryophytes at the surface and in the diaspore bank is correlated with vegetation type. We tested whether relations between above- and below-ground vegetation change in

time and tried to relate eventual changes to main seasonal environmental variations (climate, cover of vascular plants) and the presumed life-strategies of species.

Methods

Study sites

Field work was conducted in grasslands on dolomite rock in the Buda Mountains, Hungary (Plate 1). Two open grasslands were selected on southeast facing slopes of Odvas-hegy (Site 1; 47°28'05”N, 18°56'53”E) and Kő-hegy (Site 2; 47°27'56”N, 18°57'26”E). On the northwest-facing slope of the latter, a closed grassland (Site 3; 47°27'56”N, 18°57'26”E) was chosen to investigate differences between the two vegetation types.

Sampling and cultivation

Sampling dates were chosen taking main spore dispersal periods and seasonal characteristics of the habitats into consideration. Sampling was performed three times: 20–30 August 2001, 10 February 2002 and 5–6 June 2002.

A list of all bryophytes that occurred at the study sites and in their immediate surroundings was established (Appendix 1).

In order to make sampling reproducible, a permanent, 20 × 20 m grid of 5 × 5 m squares was established at each site. Above-ground vegetation was recorded in twenty-five 0.5 × 0.5 m plots at each intersection of the grid, within which total cover of bryophytes and vascular plants was estimated. The frequency of each bryophyte species was also recorded (number of 0.5 × 0.5 m plots in which the species occurred/25). No estimation was made in February 2002, since the desiccated state of the bryophytes made finding of all plants and identification in the field impossible. The diaspore bank was sampled at each site by taking duplicates of soil samples (ca. 200 cm^3 each) close to each intersection of the grid with a root auger (cf. Hock et al. 2004).

Soil samples were spread out on a layer of autoclaved wet perlite in closed plastic boxes (Plate 2). To prevent contamination with propagules originating from the surface, only the inner part of the soil cores was used. Boxes were incubated at room temperature (18-25 ºC) and under ca. 1000 µmol PAR m^{-2} s^{-1} (natural light completed by a dazzle lamp to avoid seasonal differences in illumination) in a greenhouse for 3.5 months (for details cf. Hock et al.

2004). Contamination by air-borne propagules was screened using control boxes with autoclaved, moistened perlite and autoclaved soil layer respectively, but no bryophytes were observed in these boxes.

Screening and identification

Identification and estimation of total cover of bryophytes emerging from the soil samples took place after 3.5 months. Similarly to relevés, the frequency of each species was established at each sampling date (number of soil samples in which the species occurred/total number of plots). Nomenclature of species follows Smith (2004) for mosses and Grolle (1983) for liverworts.

Deformations in the appearance of the bryophytes caused by cultivation in closed boxes, and the sterile state of most plants made identification to the species level of some individuals difficult or impossible (Hock et al. 2004). Therefore, the following groups of species were created: *Bryum* species bearing reddish rhizoid gemmae were assigned to the *Bryum erythrocarpum* agg. and *Bryum* plants with contorted leaves to the *Bryum capillare* agg. With only a few exceptions *Weissia* species found in soil samples turned out to belong to *W. controversa* during further cultivation of representative samples. *Encalypta* species occurring at open sites were assigned to *E. vulgaris*, the most frequent species in this habitat type, and, for similar reasons, those occurring in the closed grassland to *E. streptocarpa*. Species of the following genera could only be identified to the genus level in the soil samples: *Enthostodon*, *Pottia*, *Riccia*, *Tortella* and *Syntrichia*. Because of identification problems, species belonging to the genera *Barbula*, *Didymodon* and *Pseudocrossidium* are referred to as "*Barbula*" spp. *Enthostodon* plants found at the surface probably belong to *E. muhlenbergii* or *E. pulchellus*, both frequent in grasslands. However, due to their vegetative state, they could not be assigned with certainty to either of these species.

Voucher specimens have been deposited in the author's herbaria. Species were assigned to the life-strategy categories established by During (1979, 1992) following Orbán (1983), Vojtkó (1998) and Roads and Longton (2003) in the case of *Phascum cuspidatum*.

Data analysis

Part of the analysis is based on the assumption, that cover in cultivated soil samples can be used as a measure of diaspore abundance in the soil. Microscopical analysis of sieved soil

and cultivation of soil samples are the two main methods used to estimate the abundance of diaspores in the soil. However, neither of these methods provides perfect results. In the first case, it is often impossible to distinguish between soil particles and gametophyte fragments and additionally it is unclear whether these fragments are still viable. Given the large number of samples in the present study, the less time-consuming cultivation of soil samples was prefered. Drawbacks of this method include the different germination requirements of propagules and variation in the number of gametophytes produced by one propagule. In order to reduce these variations, cultivation conditions were standardized. However, it must be stated that in case of samples taken at different times of the year, the effect of conditions in the field may have influenced germination as well. Therefore, cover values obtained in soil samples were only used as an estimate of diaspore abundance in the soil when comparing differences in the total cover of bryophytes. Comparison of the abundances of individual species was based on frequencies, where the above-mentioned phenomenon does not significantly bias the results.

Statistical analyses were performed using SYN-TAX (Podani 2001) and SPSS 9.0 program package (SPSS 1999) for multivariate and univariate analyses respectively.

Differences in the species composition of open and closed vegetation types were analysed with a non-metric multidimensional scaling using the Podani's measure of discordance (Podani 2000). This method was performed both for data from soil samples and for data from the surface.

The effect of sampling date on the total cover of bryophytes was tested with different methods for the surface and soil samples. Normality of the data was tested using the Kolmogorov-Smirnov one-sample test. Data from the surface were compared with a paired t-test for data with normal distribution, and nonparametric Wilcoxson sign-ranked test for non-normal distributed data. Data from the diaspore bank were compared using repeated measures ANOVA for data with normal distribution, and nonparametric Friedman ANOVA for non-normal distributed data. In case of significant results, the significance of the differences between each sampling date was additionally tested with the parametric Bonferroni, and the nonparametric Dunn tests.

To test for differences in the total cover of bryophytes of the two vegetation types we used a Mann-Whitney U-test. Since the total cover values of vascular plants were normally distributed, differences in total cover values between the two habitat types were tested with a paired t-test.

Seasonal patterns in the relationship between the propagule bank and above-ground populations of individual species were explored by comparing their frequencies at the surface and in the diaspore bank for data from August 2001 and June 2002. For this purpose the Fisher exact test was used.

On the basis of the relationship between above- and below-ground frequencies of bryophyte species the following four groups were created. Group 1: indifferent species; frequency at the surface and in the diaspore bank not significantly different ($p>0.05$). Group 2: species significantly more frequent in the diaspore bank than at the surface ($p<0.05$). Group 3: species only present at the surface. Group 4: species only present in the diaspore bank. No group containing species more frequent at the surface than in the diaspore bank could be established.

Results

Species composition and life-strategy spectra of the two grassland types

Species composition of the two open sites was similar, whereas the closed grassland formed a distinct group both at the surface and in the diaspore bank (Fig. 1).

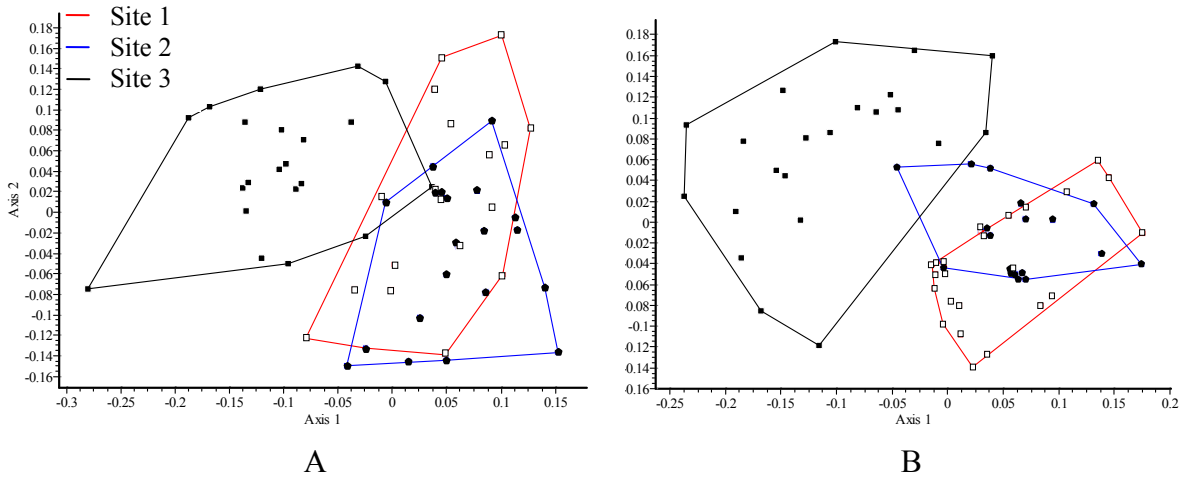


Fig. 1 Non- metric multidimensional scaling of relevés **A**) and soil samples **B**). Differences in the species composition of open and closed vegetation types. Dissimilarity function: Podani´s measure of discordance, ST: 0.1232 , ST: 0.1290 respectively.

Open grasslands – Species lists from the two sites have been united, since differences in the species composition of these two sites were minimal. From 24 species registered above ground, 20 were present in soil samples as well, while the remaining 4 species did not appear in the soil cores.; a further 3 species were only detected in soil samples.

	Open sites		Closed site	
	Relevés	Soil samples	Relevés	Soil samples
Colonist (C)	71	65	61	56
Short lived shuttle (SL)	17	17	8	11
Annual shuttle (AS)	8	9	0	7
Perennial (P)	4	9	31	26

Table 1 Unweighted life-strategy spectra of the two grassland types above-ground and in soil samples (%).

Percentages of life strategy types were similar for relevés and soil samples (Table 1). The dominating colonists were mainly represented by acrocarps with the exception of the liverwort *Cephaloziella divaricata*, which was only found in soil samples. Shuttle strategists were also important, whereas perennials were weakly represented (Table 1).

Closed grassland – During the term of the study 26 species were registered in the relevés, of which 20 were present in the soil samples; a further 7 species were only registered in the diaspore bank.

Life strategy patterns were, again, similar in both relevés and soil samples (Table 1). Similarly, the proportion of colonists was highest, however perennials were also well represented. Shuttle strategists were slightly less important than at the open sites.

Effect of seasons and habitat type on total cover of bryophytes

Total cover of bryophytes varied seasonally at all sites (Table 2). At the surface, it was significantly less in June 2002 than in August 2001 in all three grasslands. In February 2002, it was so low that estimation of the cover percentages was impossible. In contrast, in soil samples from the closed grassland total cover significantly increased between August 2001 and June 2002. An increase was also observed in both grassland types between August 2001 and February 2002.

	Open sites		Closed sites	
	Mean (%)	SD	Mean (%)	SD
Surface				
Aug. 2001	8^{a}	0.044	19^{a}	0.149
June 2002	3^{b}	0.034	12^{b}	0.174
Diaspore bank				
Aug. 2001	92^{a}	0.073	42^{a}	0.218
Feb. 2002	97^{b}	0.046	86^{bc}	0.224
June 2002	91^{ab}	0.216	91^{c}	0.149

Table 2 Effect of sampling date on the total cover of bryophytes. Different letters show significant differences ($p \leq 0.05$).

	Relevés	*Soil samples*
August 2001	open<closed	open>closed
February 2002	no data	no data
June 2002	open<closed	open>closed

Table 3 Effect of habitat type on the total cover of bryophytes. < and > represent significant differences. All significant variables have $p \leq 0.05$.

At the surface, the total cover of bryophytes (Table 3) and vascular plants (Site 2: mean = 49, SD = 17.073; Site 3: mean = 73, SD = 14.808; t = -7.218, df = 94, $p<0.001$) was significantly lower in open sites than in the closed grassland. For bryophytes, the opposite was observed in soil samples (Plate 3).

Species composition and frequencies of the species

Open grasslands – General species composition of the four groups was similar for the two open sites in August 2001 (Fig. 2). Differences detected were mainly due to the additional presence or absence of some of the more rare species. *Bryum erythrocarpum* agg., *Encalypta vulgaris*, *Weissia controversa* and *Tortella* spp. were indifferent at both sites

(Group 1). Group 2 included *Bryum argenteum* and *Phascum cuspidatum* at both sites. Species only present at the surface (Group 3) were mainly represented by epilithic acrocarps complemented by the pleurocarpic *Hypnum cupressiforme*. Group 4 comprised *Riccia* sp. at both sites, with two other, rarely found species at Site 2.

In samples taken in June 2002, most species formerly belonging to the indifferent group appeared in Group 2, or even in Group 4. Two liverwort species (*Cephaloziella divaricata* and *Mannia fragrans*) formerly present at the surface, were only detected in the soil samples. A similar pattern can be described for the formerly indifferent "*Barbula*" species at Site 1, while *Weissia controversa* switched from Group 1 to Group 2. At Site 2, these species remained in the same group over the sampling dates.

Closed grasslands – In samples from August 2001, the species frequent in both grassland types generally showed the same pattern as described above, however, some colonists and shuttle species belonging to Group 1 or 2 in open grasslands, switched to Group 2 or 4 respectively at the closed site (Fig. 3).

Compared to open grasslands, the proportion of indifferents (Group 1) increased mainly due to the additional presence of perennials, however, the colonist *Fissidens dubius* also belonged to this group. Group 2 included *Bryum erythrocarpum* agg., indifferent at the open sites. Species only present at the surface (Group 3) were, here again, represented by epilithic species and some more rare pleurocarps. Composition of Group 4 was very different from that in the open grasslands: it included *Bryum argenteum* and *Phascum cuspidatum* (belonging to Group 2 at the open sites) and rare pleurocarps.

Similar to the open sites, in June 2002 several species switched from Group 1 to Group 2 (e.g. *Weissia controversa*) or from Group 2 to Group 4 (e.g. "*Barbula*" spp.). However, in contrast to the open sites, species of the *Bryum erythrocarpum* remained in the same group. Pleurocarpic species generally showed unchanging patterns.

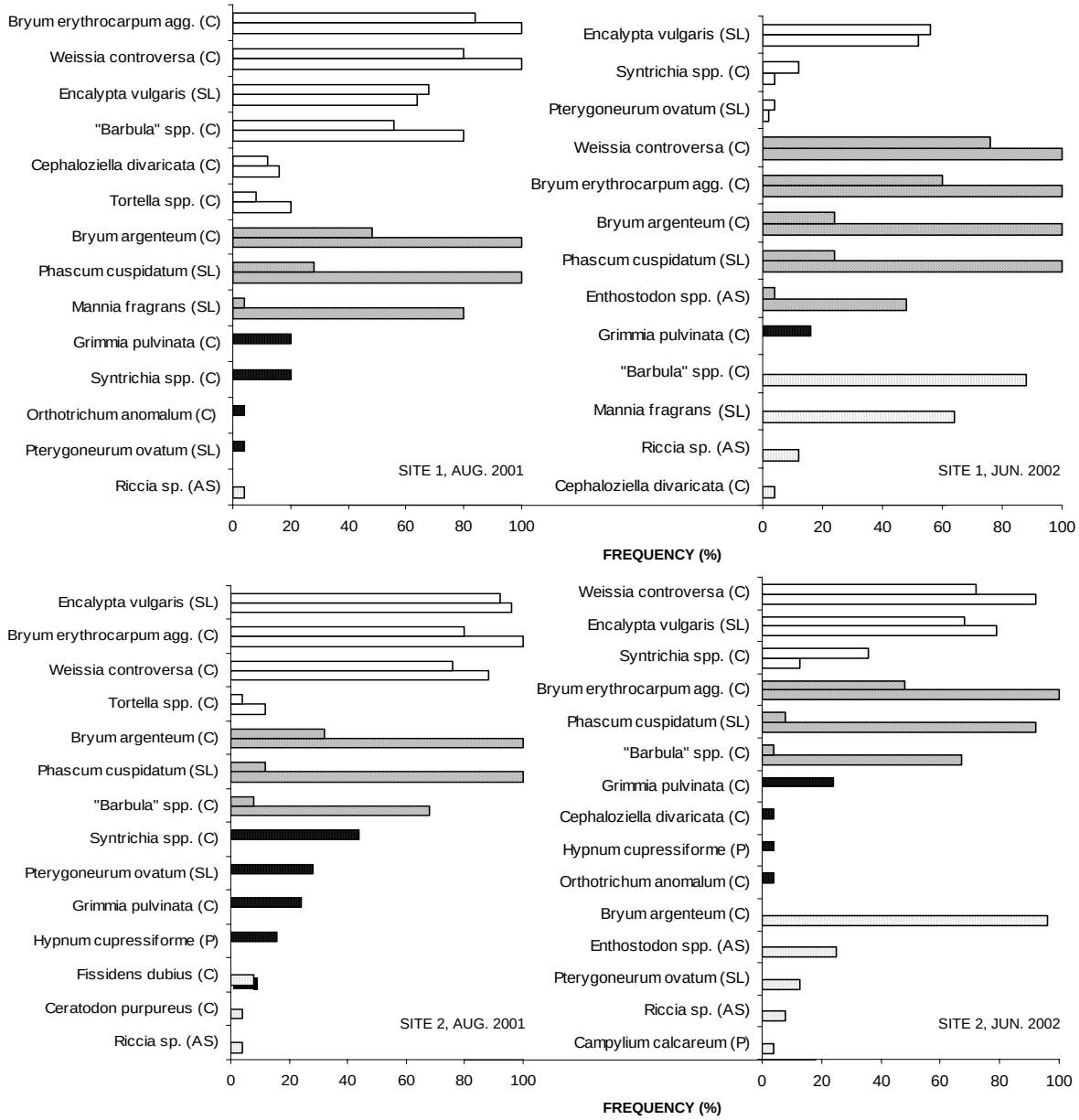


Fig. 2 Frequencies of species above-ground and in soil samples in August 2001 and June 2002, open grasslands. In the case of double columns, upper ones represent frequencies above-ground, lower ones those in soil samples. ☐ Indifferent species (Group 1), ▨ Species more frequent in the diaspore bank (Group 2), ▩ Species only present at the surface (Group 3), ⊡ Species only present in the diaspore bank (Group 4).

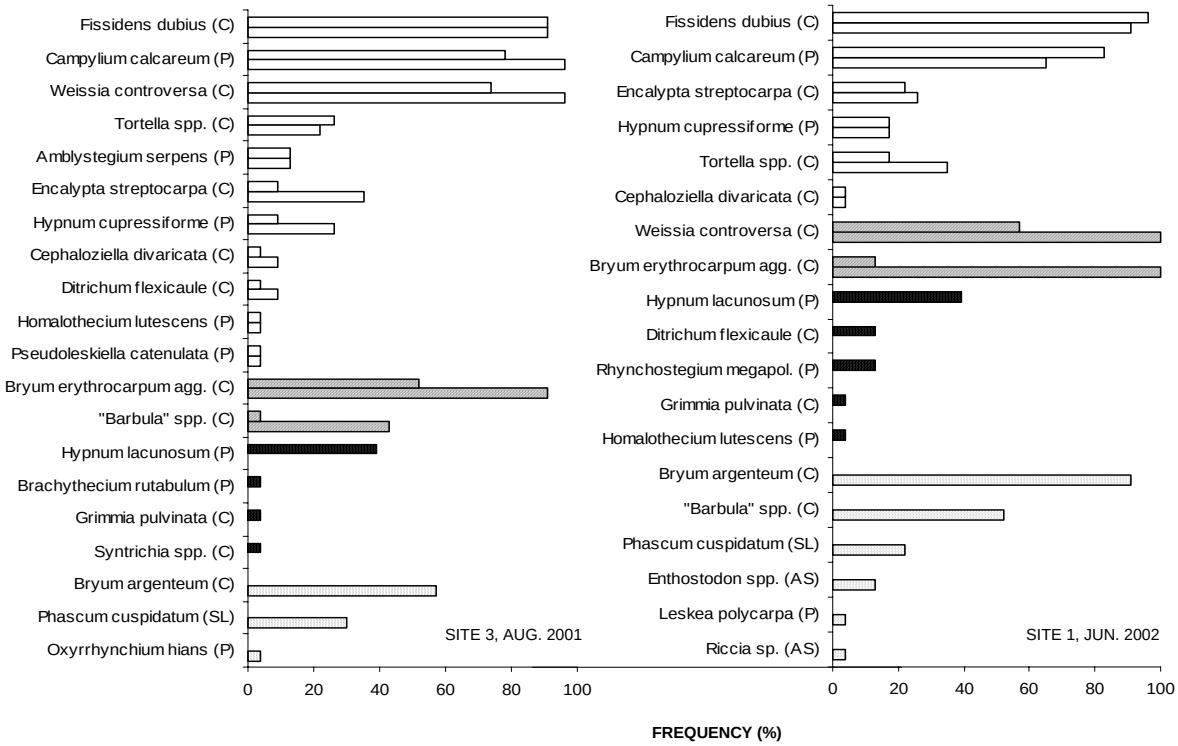


Fig. 3 Frequencies of species above-ground and in soil samples in August 2001 and June 2002, closed grassland. In the case of double columns, upper ones represent frequencies above-ground, lower ones those in soil samples.

Distribution of the life-history strategies between the four groups

Species with similar life strategies showed similar patterns concerning the distribution of life strategy types between the four groups (Table 4). Colonists occurred and were

	Site 1	Site 2	Site 3
Aug. 2001			
Group 1	***C 56*** (5), ***SL 25*** (1)	***C 33*** (3), ***SL 33*** (1)	***C 55*** (6), ***P 63*** (5)
Group 2	***C 11*** (1), ***SL 50*** (2)	***C 22*** (2), ***SL 33*** (1)	***C 18*** (2)
Group 3	***C 33*** (3), ***SL 25*** (1)	***C 22*** (2), ***SL 33*** (1), ***P 100*** (1)	***C 18*** (2), ***P 25*** (2)
Group 4	***AS 100*** (1)	***C 22*** (2), ***AS 100*** (1)	***C 9*** (1), ***P 12*** (1), ***SL 100*** (1)
June 2002			
Group 1	***C 14*** (1), ***SL 50*** (2)	***C 25*** (2), ***SL 33*** (1)	***C 40*** (4), ***P 33*** (2)
Group 2	***C 43*** (3), ***AS 50*** (1), ***SL 25*** (1)	***C 25*** (2), ***SL 33*** (1)	***C 20*** (2)
Group 3	***C 14*** (1)	***C 38*** (3), ***P 50*** (1)	***C 20*** (2), ***P 50*** (3)
Group 4	***C 29*** (2), ***AS 50*** (1), ***SL 25*** (1)	***C 12*** (1), ***AS 100*** (2), ***SL 33*** (1), ***P 50*** (1)	***C 20*** (2), ***AS 100*** (2), ***SL 100*** (1), ***P 17*** (1)

Table 4 Distribution of life strategy types between the four groups. AS – annual shuttle, C – colonist, F – fugitive, P – perennial, SL – short lived shuttle. Values in bold italics represent the percentage of the given strategy type occurring in each group. Values in parentheses indicate the number of species belonging to the given strategy type.

dominant in almost all groups at both sampling dates. The most frequent shuttle species did not switch groups between the two sampling dates, with the exception of *Mannia fragrans*, which passed from Group 2 to Group 4 in June 2002.

The closed grassland had a different distribution of life strategy types (Table 4). Colonists were still the most frequent and occurred in all groups. However, perennial pleurocarps were important as well. Nevertheless, these species were lacking from Group 2. In contrast to the open sites, the only short-lived shuttle species, *Phascum cuspidatum*, only occurred in the diaspore bank at both sampling dates. The two annual shuttle taxa, *Riccia* sp. and *Enthostodon* spp. were only registered in soil samples from June 2002.

Discussion

Species richness and life-strategies

The number of species found at the surface and in the diaspore bank of both habitat types were fairly high compared to other studies in similar habitats (Bisang 1996; During et al. 1987). Similar to vascular plants (Bartha et al. 1998; Fekete and Kovács 1978), species number was slightly higher in the closed grassland. This can clearly be explained by the additional presence of perennial species in this habitat. Whereas colonists, typical for the open sites, frequently appeared in open patches of the closed grassland, only a few perennials tolerated the extreme conditions found in the open habitats. In addition, some colonists, such as *Cephaloziella divaricata*, *Encalypta streptocarpa* and *Fissidens dubius* prefered closed grasslands (Rincon and Grime 1989; Watson 1960).

Observed dominance of colonists in both grassland types is congruent with previous findings (During and ter Horst 1983; During et al. 1987). These short-lived species allocate great amounts of energy in reproduction, and thus exploit short favourable periods efficiently (During 1979). In open grasslands, temporary gaps are formed continuously. Colonists can rapidly colonize these gaps by means of their numerous, mostly long-lived propagules "waiting" in the soil. In contrast, perennials are underrepresented in this habitat since they are adapted to longer-lived microhabitats with generally more constant conditions, and their reproductive investment is also much lower. In closed grasslands, however, they are able to maintain large colonies due to the considerable growth and longer life-span of shoots.

Similarly to colonists, shuttle strategists exploit gaps and generally produce numerous, mostly long-lived propagules. In this study, they occurred at both grassland types, but were less important in the closed site where most of them were found in the diaspore bank, probably due to the limited number of gaps available.

Effect of habitat type and seasons on total cover of bryophytes

In contrast to our findings, Virtanen et al. (2000) reported declining bryophyte cover with increasing cover of vascular plants, which was ascribed to the less effective competitive ability of bryophytes. In our case, this is only present at the level of the life strategy: perennials, as better competitors dominate in the closed habitat. The total cover of bryophytes may correlate with the total cover of vascular plants as well as with their growth form and

architecture (Watson 1960). In Site 3, rosette plants, unfavourable for bryophyte growth, are lacking and dominant grass species (*Bromus pannonicus* Kumm. & Sendtn., *Festuca pallens* (L.) Holub., *Stipa* spp.) are narrow-leaved. This kind of habitat provides nearby optimal conditions for bryophyte growth (Watson, 1960), which may explain the high cover values obtained there. In contrast, in the open grasslands sparsely occurring, extremely short turfs, steepness of the slopes and southern exposition all contribute to shortage of water, which is usually connected with the sparse occurrence of bryophytes (Watson, 1960).

Considering the life cycle and growth form of the dominant life-strategies at the two habitat types, the picture becomes even clearer. In open grasslands, the dominating colonists and shuttle species are only present at the surface for short periods of time, and hence they may be absent at the time of sampling. Moreover, most of them are acrocarps forming short turfs of erect shoots, instead of extended mats. The large number of long-lived propagules produced by such species accumulate in the soil (During 1979; During et al. 1987). These may be at the origin of the low total cover values at the surface and the high total cover obtained in soil samples. In contrast, perennials, typical of the closed site, allocate more energy into growth (During 1979; During et al., 1987). They are mostly pleurocarps, forming large mats constantly present at the surface but they usually lack specialized vegetative propagules, reproduce late and their spores are mostly short lived (During 1992). This explains high cover values at the surface and low values in soil samples at the closed site.

Cover of bryophytes in grasslands varies seasonally: it reaches a peak in moist, cool seasons and decreases in summer (e.g. Al-Mufti et al. 1977). This is in accordance with our results, where the above-ground cover was highest in early autumn and decreased significantly towards summer. Data from soil samples complement these results, with lowest cover values in August 2001 and a significant increase in February and June 2002. Sampling in August 2001 was carried out approximately 10 days after the beginning of autumn rains and milder temperatures. These conditions probably allowed for the regeneration of bryophytes after the summer drought, and allowed new shoots to arise from the diaspore bank (Keizer et al. 1985; Rincon and Grime 1989), which also partly explains the decrease obtained in the soil samples. The low cover in the field in February 2002 may be due to the dry and very cold period, which preceded sampling and probably increased shoot mortality. It is important to remark, that many of the brown, seemingly dead shoots still contain viable cells, thus this increased shoot mortality may provide the diaspore bank with additional vegetative propagules. The humid and mild period between February and June 2002 was again favourable for bryophyte growth, which could again have caused an increase in their cover,

however, preceding the sample date, the weather changed and the study sites experienced a few, extremely dry and hot weeks. These probably had a similar effect on the bryophyte cover and shoot mortality as would winter frosts. These hypotheses are supported by the increasing amount of propagules in soil samples.

Distribution of life strategies between the four groups

The fact that no species more frequent in relevés than in soil samples bank have been found suggests that, with the exception of some rare perennials, bryophytes occurring in grasslands generally rely upon a considerable diaspore bank, as discussed later on.

When looking at the composition of the four groups, the first outstanding issue is that species belonging to the same life strategy type appeared in different groups at the same time of the year. This is especially true for colonists. In open sites colonists dominated almost all groups. Their presence in Group 1, 2 and 4 is not surprising, since they appear at the surface for short periods only, but their propagules, produced in large amounts, are accumulated in the soil (During 2001). It is more surprising that the frequently sporulating epilithic colonists *Grimmia pulvinata* and *Orthotrichum anomalum* were never detected in the soil samples. Since the latter was fairly rare it will not be dealt with in the further discussion. The strictly epilithic *Grimmia pulvinata* lacks vegetative propagules but regularly and abundantly produces spores in late spring. Several authors reported difficulties when trying to germinate spores of the genus *Grimmia* (Keever 1957; Longton and Miles 1982). Spores were not dormant since protonemal filaments developed, but no buds were formed afterwards. H.J. During (pers. comm.) only observed gametophytes on protonemata of the species, when these were left desiccated for about a year. *Leptobarbula berica*, another epilithic species, was never seen growing on the soil itself in cultivated soil samples, but rather only on small pieces of chalk (H.J. During pers. comm.). It is thus possible, that either the substrate used for the cultivation of soil samples was not appropriate for *G. pulvinata* or that it was present in the samples but only at the protonemal stage, which rendered detection impossible. We hypothesize that strictly epilithic species may establish only on stony substrate and need much time and/or serious desiccation to form gametophytes.

Annual shuttle species exploit short-lived gaps: they have a short life cycle and large, long-lived spores capable of bridging longer unfavourable conditions. Accordingly, taxa representing this group (*Enthostodon* spp. and *Riccia* sp.) were mainly found in the diaspore bank.

Short-lived shuttles have longer life cycles but still strongly rely upon the diaspore bank (During 1992, 2001), as confirmed by our results. *Encalypta vulgaris* remained in the indifferent group over the sampling dates, which indicates that in addition to a large diaspore bank, the species is also constantly present above ground. In contrast, the liverwort *Mannia fragrans*, which also forms a large diaspore bank, disappeared from the surface in June 2002. Since it is almost impossible to find thalli when they are in an enrolled state it is possible that they were overseen in June 2002. *Phascum cuspidatum*, a short-lived (or even long-lived) shuttle species has perennating shoots and its spores are thought to fail to germinate in the field (Roads and Longton 2003). Results confirm the great role of the diaspore bank in the life cycle of this species, but gametophytes originating from buried plant fragments were rarely found during microscopical inspection.

Perennials were underrepresented in open grasslands. *Campylium calcareum*, only found in soil samples, is abundant in neighbouring closed sites. It produces no sporophytes at the study sites and has no special asexual propagules, which may limit its colonizing ability at the open sites. Still, some propagules may reach the open sites, but the arid conditions present there probably hamper germination. *Hypnum cupressiforme*, a widely tolerant species, also prefers closed grasslands. Although it produces no sporophytes at the sites investigated, it frequently sporulates in the surroundings, and its vegetative reproduction is also efficient (Szövényi 2002). Plants found at the open grassland may have originated either from vegetative fragments from the proximate closed site or from spores coming from greater distances.

Differences between open and closed grassland types can be explained by their different structure and microclimate, as described earlier. In this context, it is easy to understand that species occurring in both habitat types often belonged to another group in the closed site than in the open grasslands. The most striking trend in this respect was again observed in colonists and shuttle strategists. Species belonging to Group 1 or 2 in the open grasslands switched to Group 2 and 4 respectively (e.g. *Bryum erythrocarpum* agg., *Bryum argenteum*, and *Phascum cuspidatum*). This is probably related to the rarity of short-lived gaps in closed grasslands, which, together with the presence of better competitors (perennials) reduces the opportunities of such species to appear at the surface. However, their propagules, partly probably originating from open sites, are accumulated in the soil.

Interestingly, three colonists (*Ditrichum flexicaule*, *Encalypta streptocarpa* and *Fissidens dubius*) preferred the closed grassland. The two latter species always occurred together with perennials in shaded microhabitats. Sporophytes of both species are rare (Smith

2004), but both produce numerous asexual propagules, which is typical of "ephemeral colonists" (*sensu* During 1992). However, their life span of several years contradicts this classification. This indicates that transitions between established life history strategy types are frequent, and it is often difficult to assign a species to one of those types. One of the most heterogeneous groups was that of the colonists. *Ditrichum flexicaule*, preferred open patches, but, in contrast to other colonists, it did not form any diaspore bank. This is not surprising since the species sporulates very rarely (Smith 2004 and no sporophytes found at the study sites) and lacks specialised asexual propagules (Arts 1994). In addition, turfs of the species were constant. Hence, *D. flexicaule* seems to represent a transition towards the perennial strategy.

Perennials generally tend to be absent from the diaspore bank (During 2001). In our case, they were lacking from the group of species more frequent in the diaspore bank (Group 2). However, solitary exemplars of some perennials absent from the sampling grid (*Pseudoleskiella catenulata*) or the whole site (*Oxyrrhynchium hians* and *Leskea polycarpa*) were detected in soil samples. As propagules of bryophytes are capable of travelling longer distances (van Zanten and Gradstein 1988), these might have originated from remote sites.

Differences between sampling dates – Between August 2001 and June 2002, many species switched from the indifferent group to the group of species more frequent or restricted to the diaspore bank in both grassland types. This is probably the result of the same factors, which appear to have caused the changes observed in the total cover of bryophytes: favourable conditions allowing bryophyte growth at the surface in August 2001, followed by increased shoot mortality causing a decrease in frequency at the surface and thus providing propagule supply for the diaspore bank. The fact that some species living in habitats with seasonally occurring dry periods show enhanced production of drought-resistant asexual propagules with the approach of the dry season (Arts 1986) may have also contributed to the results obtained.

In contrast to colonists and shuttle strategists, pleurocarpic perennials generally remained in the same group, which is in accordance with their longer life cycle and more constant presence at the surface.

More expressed differences between the two sampling dates at Site 1 probably correlate with the structure of this site containing more open patches than Site 2 and thus probably being less favourable for bryophyte growth in dry seasons.

This study constitutes one of the first successful attempts to relate species composition and dynamics in the diaspore bank of bryophytes to that in the overlaying vegetation over seasons. Since above- and below-ground patterns are often tightly coupled, they should not be approached as separate systems. Our results show that parallel analysis of both can reveal important aspects related to the dynamics of individual species and the community as a whole.

Acknowledgements

The authors thank Heinjo During, Jakob Schneller and Edwin Urmi for their valuable comments on an early version of the manuscript. Merran Matthews is gratefully acknowledged for correcting the language of the manuscript.

References

Al-Mufti MM, Sykes CL, Furness SB, Grime JP, Band SR 1977 A quantitative analysis of shoot phenology and dominance in herbaceous vegetation. *J Ecol* **65**: 759–791

Arroyo MTK, Cavieres LA, Castor C, Humaña AM 1999 Persistent soil seed bank and standing vegetation at high alpine site in the Chilean Andes. *Oecologia* **119**: 126-132

Arts T 1986 Drought resistant rhizoidal tubers in *Fissidens cristatus* Wils. Ex Mitt. *Lindbergia* **12**: 119-120

Arts T 1994 Rhizoidal tubers and protonemal gemmae in European *Ditrichum* species. *J Bryol* **18**: 43-61

Bartha S, Rédei T, Szollát Gy, Bódis J, Mucina L 1998 Északi és déli kitettségű dolomitsziklagyepek térbeli mintázatainak összehasonlítása. In: Csontos P (ed) *Sziklagyepek szünbotanikai kutatása.* Scientia Kiadó, Budapest

Bisang, I 1996 Quantitative analysis of the diaspore banks of bryophytes and ferns in cultivated fields in Switzerland. *Lindbergia* **21**: 9-20

Csontos P, Tamás J, Kalapos T 1996 Soil seed banks and vegetation recovery on dolomite hills in Hungary. *Acta Bot Hung* **40**: 35-43

Csontos P, Tamás J,. Podani. J 2004 Slope aspect affects the seed mass spectrum of grassland vegetation. *Seed Sci Res* **14**: 379-385

Draskovits R, Kovács-Láng E 1968 Mikroklimamessungen in Kalkstein- und Dolomitfelsenrasen. *Ann Univ Sci Budap, Sectio Biol* **9-10**: 115-129

During HJ 1979 Life strategies of bryophytes; a preliminary review. *Lindbergia* **53**: 2-18

During HJ, ter Horst B 1983 The diaspore bank of bryophytes and ferns in chalk grassland. *Lindbergia* **9**: 57-64

During HJ, Bruguès M, Cros RM, Lloret F 1987 The diaspore bank of bryophytes and ferns in the soil in some contrasting habitats around Barcelona, Spain. *Lindbergia* **13**: 137-149

During HJ 1992 Ecological classification of bryophytes and lichens. In: Bates JW, Farmer AM (eds) *Bryophytes and lichens in a changing environment.* Oxford University Press, Oxford

During HJ, Lloret F 1996 Permanent grid studies in bryophyte communities. I. Pattern and dynamics of individual species. *J Hattori Bot Lab* **79**: 1-41

During HJ 1997 Bryophyte diaspore banks. *Adv Bryol* **6**: 103-134

During HJ 2001 Diaspore banks. *Bryologist* **104**: 92-97

Fekete G, Kovács M 1978 The space dynamism of species and life form diversity in two rocky grassland communities. *Acta Biol Debr* **15**: 7-21

Ghorbani J, Das PM, Das AB, Hughes JM, McAllister HA, Pallai SK, Pakeman RJ, Marrs RH, Le Duc MG 2003 Effects of restoration treatments on the diaspore bank under dense *Pteridium* stands in the UK. *Appl Veg Sci* **6**:189–198

Grillas P, Garcia-Murillo P, Geertz-Hansen O, Marbá N, Montes C, Duarte CM, Tan Ham L, Grossmann A 1993 Submerged macrophyte seed bank in a Mediterranean temporary marsh: abundance and relationship with established vegetation. *Oecol* **94**: 1-6

Grolle R 1983 Nomina generica hepaticarum; references, types and synonymies. *Acta Bot Fenn* **121**: 1-62

Henderson CB, Petersen KE, Redak RA 1988 Spatial and temporal patterns in the seed bank and vegetation of a desert grassland community. *J Ecol* **76**: 717-728

Hock Zs, Szövényi P, Tóth Z 2004 Seasonal variation in the bryophyte diaspore bank of open grasslands on dolomite rock. *J Bryol* **26**: 285-292

Keever C 1957 Establishment of *Grimmia laevigata* on bare granite. *Ecol* **38**: 422-429

Keizer PJ, van Tooren BF, During HJ 1985 Effects of bryophytes on seedling emergence and establishment of short-lived forbs in chalk grassland. *J Ecol* **73**: 493-504

Longton RE, Miles CJ 1982 Studies on the reproductive biology of mosses. *J Hattori Bot Lab* **52**: 219-240

Orbán S 1983 *A magyarországi mohák stratégiái, összefüggésük a környezet ökológiai és cönológiai jellemzőivel.* Dissertation, Eötvös Loránd University, Budapest, Hungary

Podani J 2000 *Introduction to the exploration of multivariate ecological data.* Backhuys Publishers, Leiden, The Netherlands

Podani J 2001 SYN-TAX 2000. *Computer program for data analysis in ecology and systematics. User´s manual.* Scientia Publishing, Budapest, Hungary

Rincon E, Grime JP 1989 Plasticity and light interception by six bryophytes of contrasted ecology. *J Ecol* **77**: 439-446

Roads E, Longton. RE 2003 Reproductive biology and population studies in two annual shuttle mosses. *J Hattori Bot Lab* **93**: 305-318

Smith AJE 2004 *The moss flora of Great Britain and Ireland.* Cambridge University Press, Cambridge

SPSS 1999 *SPSS Base 9.0 for Windows. User´s guide.* Chicago, IL: SPSS Inc

Szövényi P 2002 *The phorophyte preferences of epiphytic bryophytes in a* Carici brizoidis-Alnetum *alliance in NW Hungary* [in Hungarian with English summary]. Masters thesis. Eötvös Loránd University, Budapest, Hungary

Van Zanten BO, Gradstein SR 1988 Experimental dispersal geography of neotropic liverworts. *Nova Hedw, Beiheft* **90**: 41-94

Virtanen R, Johnston AE, Crawley MJ, Edwards GR 2000 Bryophyte biomass and species richness in the Park Grass Experiment, Rothamsted, U.K. *Plant Ecol* **151**:129-141

Vojtkó A 1998 Relationships between the morphology of the spores of Hungarian Hepaticae species and their strategy types. *Studia Bot Hung* **27-28**: 109-115

Watson EV 1960 A quantitative study of the bryophytes of chalk grassland. *J Ecol* **48**: 397-414

Appendix

Appendix 1 List of all species found in relevés (R) and in soil samples (S) at the two grassland types during the whole term of the study. AS – annual shuttle, C – colonist, F – fugitive, P – perennial, SL – short lived shuttle.

	Open sites		Closed site	
Species	R	S	R	S
Amblystegium serpens (P)			×	×
Barbula sp. *(C)*		×		×
Barbula unguiculata (C)		×		×
Brachythecium rutabulum (P)			×	
Bryum argenteum (C)	×	×	×	×
Bryum capillare agg. *(C)*	×	×	×	×
Bryum erythrocarpum agg. *(C)*	×	×	×	×
Campylium calcareum (P)		×	×	×
Cephaloziella divaricata (C)	×	×	×	×
Ceratodon purpureus (C)	×	×		
Didymodon acutus (C)	×			
Ditrichum flexicaule (C)			×	×
Encalypta streptocarpa (C)	×	×	×	×
Encalypta vulgaris (SL)	×	×	×	×
Enthostodon sp. *(AS)*	×	×		×
Fissidens dubius (C)	×	×	×	×
Grimmia pulvinata (C)	×		×	
Homalothecium lutescens (P)			×	
Hypnum cupressiforme (P)	×	×	×	×
Hypnum lacunosum (P)			×	×
Leskea polycarpa (P)				×
Mannia fragrans (SL)	×	×		×
Orthotrichum anomalum (C)	×		×	
Oxyrrhynchium hians (P)				×
Pottia bryoides (C)	×	×	×	×
Phascum cuspidatum (SL)	×	×	×	×
Pseudocrossidium hornschuchianum (C)	×	×	×	×
Pseudoleskiella catenulata (P)			×	×
Pterygoneurum ovatum (SL)	×	×		
Rhynchostegium megapolitanum (P)			×	
Riccia sp. *(AS)*	×	×		×
Syntrichia intermedia (C)	×			
Syntrichia ruralis (C)	×	×	×	×
Tortella inclinata (C)	×	×	×	
Tortella tortuosa (C)			×	×
Weissia controversa (C)	×	×	×	×
Weissia brachycarpa (C)	×	×	×	×

Plate 1 Study sites. A. Aerial photograph of the sampling area. 1. Site 1, 2. Site 2, 3. Site 3. B. Odvas-hegy, southeast slope, open grassland. C. Kő-hegy, northwest slope, closed grassland.

Plate 2 Incubation of soil samples. A-B. Soil samples in the greenhouse. C. Bryophytes emerging from the soil samples.

Plate 3 A. Soil sample from an open grassland with numerous plants emerging. B. Soil sample from the closed grassland with scattered plants.

Chapter II.

Seasonal variation in the spore bank of ferns in grasslands on dolomite rock

Zsófia Hock[1,2], Péter Szövényi[1,2] and Zoltán Tóth[2]

[1]*Institute of Systematic Botany, University of Zürich, Zürich, 8008, Zollikerstr. 107, Switzerland;*
[2]*Eötvös Loránd University, Budapest, 1117, Pázmány Péter s. 1/C, Hungary*

Published in

Plant Ecology

Abstract

Composition and seasonal patterns of the fern spore bank were compared to the surface vegetation of grasslands on dolomite rock in Hungary. Viability and potential dormancy of spores were tested through storage experiments. Although *Asplenium ruta-muraria* L. was the only species found at the study sites, five others, probably originating from airborne spores from nearby areas, emerged from the soil samples. Considerable seasonal variability was detected in the number of prothallia emerging from soil samples from different sampling dates, with a peak after spore dispersal. The increased number of emerging prothallia after 1 year of storage suggests that a part of the spores stored in the soil samples were presumably dormant. Investigations on the dormancy of fern spores might be of great interest, especially in species adapted to seasonally unfavourable habitats.

Keywords: Asplenium ruta-muraria; dormancy; ferns; propagule bank; seasonality

Introduction

Propagule banks play an important role in the life of plant populations: they provide a seasonal escape from environmental limitations, ensure persistence, regeneration and recolonization after disturbance and help conserving genetic variability. While the number of investigations on the propagule bank of seed plants and bryophytes has increased considerably in the last decade, covering a wide range of questions, there are still relatively few data available for the spore banks of pteridophytes. Since the presence of reservoirs of viable fern spores in the soil was first experimentally confirmed (e.g. Strickler and Edgerton 1976), existing studies have mainly focused on composition in various habitats (During and ter Horst 1983; During et al. 1987; Leck and Simpson 1987; Dyer and Lindsay 1992; Bisang 1996) or within a population of a species (Schneller 1988, 1999), on relationships to depth and to the above-ground vegetation (Bisang 1996; Rydgren and Hestmark 1997; Ramírez-Trejo et al. 2004). Long-distance dispersal turned out to be an important factor in colonization by ferns, because many studies found a rich spore bank containing species occurring far from the study sites (e.g. During and ter Horst 1983; Leck and Simpson 1987). Non-chlorophyllous fern spores are generally capable of surviving longer periods than chlorophyllous ones (Lloyd and Klekowski 1970). Since most fern species possess non-chlorophyllous spores, fern spore banks have been described as persistent (During and ter Horst 1983). Seasonal variations in the fern spore bank have only been occasionally studied (During and ter Horst 1983; Dyer and Lindsay 1992; Penrod and McCormick 1996; Ranal 2003). During and ter Horst (1983) found that the number of plants emerging from soil samples reached its maximum following spore dispersal (During and ter Horst 1983). This might be a widespread pattern, but no general conclusion can be made due to the low number of studies available. Nevertheless, variations in the number of propagules germinating from soil samples collected at different times of the year can also be due to other factors, including dormancy of spores (*sensu* Raghavan 1989; Lloyd and Klekowski 1970; Spiess and Krouk 1977; Hock 2003). Spore dormancy is also a poorly investigated field of fern biology, although physiological dormancy has been reported in some cases (reviewed in Raghavan 1989 and Dyer and Lindsay 1992).

The first aim of our research was to detect seasonal patterns of the fern spore bank in habitats with periodically unfavourable temperature and moisture conditions. We aimed to test to what extent spore dispersal periods of the most common species and seasonally varying environmental conditions influence the composition of the spore bank. Finally, information

was collected about the viability and potential dormancy of fern spores, both factors potentially influencing the number of plants emerging from the soil samples.

This study is part of a series of investigations on the diaspore bank of bryophytes and ferns in different habitat types, including grasslands on dolomite rock in Hungary (Hock 2003).

Methods

Study sites

The study area is situated in the Buda Mountains (ca 250–350 m a.s.l.), southwest of Budapest, Hungary. Special physical and chemical characteristics of the dolomite bedrock and steepness of the slopes result in a microclimate with great daily variations in temperature and moisture conditions; thus, these hills mainly support a xero- and thermophilous flora (Zólyomi 1942; Draskovits and Kovács-Láng 1968; Dobolyi et al. 1991; Dobolyi 1997, 2002). Three neighbouring sites were selected for the study (Plate 1).

Site 1 and 2 were situated on neighbouring southeast-facing steep slopes of Odvas-hegy (47°28'05" N, 18°56'53" E) and Kő-hegy (47°27'56" N, 18°57'26" E). The vegetation at both sites belongs to open grassland (*Seseli leucospermi- Festucetum pallentis*; Zólyomi 1958), characterized by a very shallow soil layer and the dominance of ephemeral and annual plants.

Site 3 is found near to site 2, on the northwest-facing slope of Kő-hegy (47°27'56" N, 18°57'26" E). Owing to its exposure, this site has more constant microclimatic conditions, which allows the development of a closed grassland vegetation (*Festuco pallenti-Brometum pannonici*; Zólyomi, 1958) with perennials dominating.

All sites have two main annual growth periods: one in spring, followed by an extremely warm and dry summer with little or no growth, and another in autumn.

Sampling

Sampling dates were chosen taking into consideration seasonal characteristics of the habitat and spore dispersal periods of the most frequent Hungarian fern species (July–September; Simon 2000). Sampling was thus carried out at the following dates: 1. 20–30 August 2001; 2. 10 February 2002; 3. 5–6 June 2002.

Sampling occurred within a randomly established permanent plot (20×20 m grid of 5×5 m squares) at each site. Position of all plots has been marked both in the field and on a map. On each date two soil cores (area *ca* 200 cm^3; depth *ca* 3 cm) were taken close to each intersection using a root auger (altogether 150 samples/sampling date). The samples were transported to a greenhouse in closed plastic bags. Fern species in the surface vegetation of all sites were recorded at every sampling date.

Cultivation and analysis of soil samples

To avoid contamination (Schneller 1999), only a thin layer (few millimetres) of the inner part of the soil cores was sowed out (n=150/sampling date) on a layer of autoclaved, wet perlite, in closed, transparent plastic boxes (*ca* 10×10 cm). In a first step, the samples were cultivated in a greenhouse for 3.5 months at room temperature (average: 22–23ºC, regulated to compensate outside temperature changes) and under ca. 1000 µ mol PAR m^{-2} s^{-1} (natural light completed by a lamp to compensate seasonal differences). This first step was necessary because the bryophyte diaspore bank was analysed at the same time, and many bryophytes were overgrown by algae after longer cultivation. For this reason, besides conserving the original samples, a representative sample of fern prothallia showing morphological differences was transplanted into plastic containers and cultivated until identification became possible (generally 2 years). Control samples containing autoclaved perlite and soil layers were used to detect possible contamination from air-borne spores. Plastic boxes were protected from direct sun and regularly randomly rearranged to avoid effects of differential light conditions.

To test the longevity of fern spores and to collect information about the role of a potential spore dormancy, each soil sample collected in August 2001 (only from sites 2 and 3, n=50/site) was mixed and subdivided into three equal proportions, of which one was immediately spread out (cf. above). The second and the third part (50–50 samples each) were air-dried and stored in the laboratory at temperatures between 15 and 23ºC. These were spread out after 6 and 12 months, respectively. Cultivation of these samples occurred in the same way as described above.

At the first screening, after 3.5 months, the presence of prothallia was recorded within a 10×10 cm grid of 1×1 cm squares using a dissecting microscope. Data are presented as frequency of occurrence in grid cells. However, the results obtained approximate well the exact number of prothallia found, since one square of the grid was usually occupied by one prothallium, squares containing several prothallia occurred very rarely. Identification of the species took place after ca 2 years of cultivation. Separation of morphological groups at the prothallium stage followed Stokey (1951), Herrero et al. (2002) and Schneller (1975). Since all plants could not be identified to the species level at the end of the cultivation period, densities for individual species are not given. Nomenclature of the species follows Simon (2000).

Data analysis

Normality of the data was tested using the Kolmogorov-Smirnov one-sample test (Sokal and Rohlf 1995).

The effect of sampling date and duration of storage on the number of prothallia emerging from the soil samples were tested with a repeated measures ANOVA (Sokal and Rohlf 1995) for data with normal distribution, and nonparametric Friedman ANOVA (Sokal and Rohlf 1995) for non-normal distributed data.

Where significant results were achieved, the significance of the differences between each sampling date was additionally tested with the parametric Bonferroni, and the nonparametric Dunn tests (Sokal and Rohlf 1995).

All statistical analyses were performed using the SPSS 9.0 program package (SPSS, 1999).

Results

Results of the statistical analyses are presented in Table 1.

Species composition above-ground and in soil samples

Ferns proved to be very rare in the vegetation at all study areas. The only species found aboveground was *Asplenium ruta-muraria* L. that occurred only in three rock crevices at Site 1.

In contrast, a great number of prothallia (ca 10–30/200 cm^3 soil) of several fern species emerged from the soil samples of all sites (Plate 4). At the first screening date, gametophytes could already be divided into three morphological groups. A large number bore unicellular papillate hairs of two distinct forms: small, cylindrical, almost triangular hairs and club-shaped hairs. Prothallia with triangular hairs, typical of several *Asplenium* species (including *A. ruta-muraria*, but not *A. trichomanes*), dominated the samples, while club-shaped hairs (*Dryopteris* spp.) occurred only rarely. The third group were naked prothallia that were also uncommon (*Athyrium* spp.).

After 2 years of cultivation, the following six species could be identified: *Asplenium ruta-muraria* (about 90% of all prothallia), *A. trichomanes* L., *A. ceterach* L., *Athyrium filix-femina* (L.) Roth, *Cystopteris fragilis* (L.) Bernh. and *Dryopteris filix-mas* (L.) Schott. Two additional taxa, belonging either to *Asplenium* or to *Dryopteris*, could not be identified to the species level.

Changes in the frequency of prothallia between sampling dates

In general, soil samples from open sites (Site 1 and 2) showed similar trends (Fig.1). In samples taken from these two sites (Site 1 and 2) during February 2002, the frequency of emerging prothallia was significantly lower than in samples from August 2001. However, a general increase in the frequency of the prothallia could be observed in the soil samples from June 2002 at both sites. The frequency of prothallia also increased between August 2001 and June 2002 at site 1 but decreased at site 2.

In the closed grassland (Site 3) differences were only significant between August 2001 and June 2002 (Table 1).

	Repeated measures or Friedman ANOVA		Bonferroni or Dunn post-hoc test		
	MS/Fr	P	(August/February)	(August/June)	(February/June)
Effects of seasonal sampling					
Site1	***0.185***	***<0.0001***	***P<0.01***	***P<0.05***	***P<0.001***
Site2	*29.621*	*<0.0001*	*P<0.001*	*P<0.05*	*P<0.05*
Site3	***0.082***	***0.0007***	***P>0.05***	***P<0.001***	***P>0.05***
	Friedman ANOVA		Dunn post-hoc test		
	Fr	P	(Control/6 months)	(Control/12months)	(6months/12months)
Effects of storage					
Site2	*42.091*	*<0.0001*	*P<0.01*	*P<0.01*	*P<0.001*
Site3	*28*	*<0.0001*	*P>0.05*	*P>0.05*	*P<0.001*

Italics: Friedman ANOVA, Bold italics: Repeated measures ANOVA

Table 1 Results of the statistical analysis.

Effect of storage time on the frequency of prothallia

Compared to the soil samples stored for 6 months, significantly more prothallia emerged from the soil samples from both sites 2 and 3 after 1 year (Fig. 2). In the case of the open grassland (Site 2), compared to the samples spread out immediately, the frequency of emerging prothallia significantly decreased after 6 months of storage, but increased again after 1 year. Similar changes were recorded for the closed grassland samples (Site 3), but only the increase after 1 year of storage turned out to be significant.

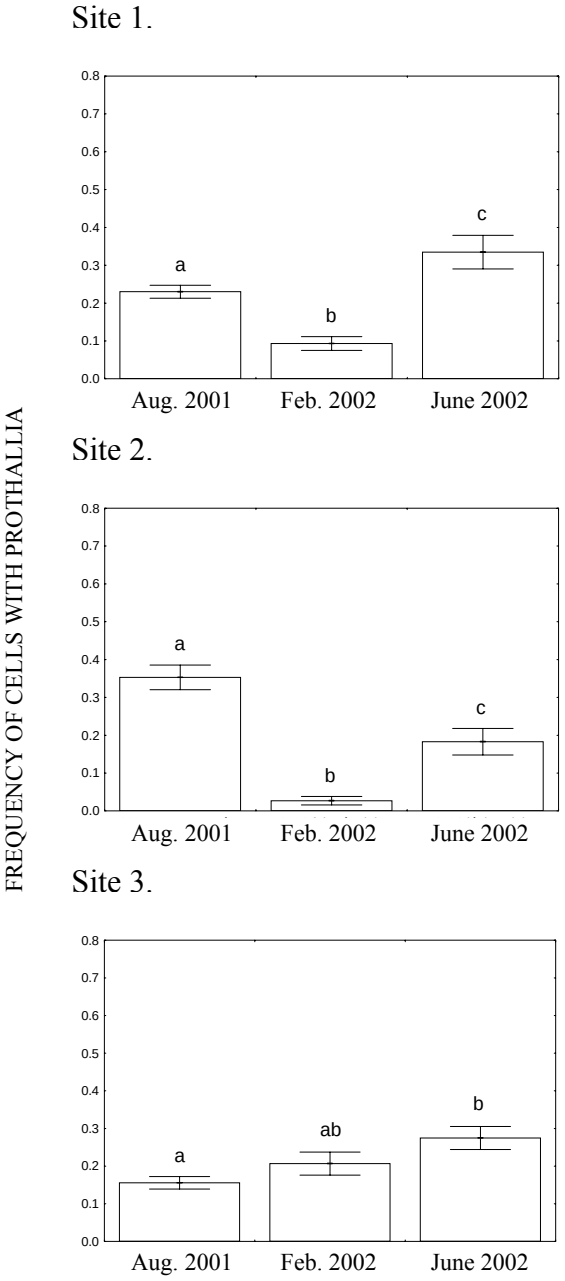


Fig. 1 Effect of seasonal sampling on the frequency of prothallia emerging from the soil samples (n=50). The different letters represent significantly (P≤0.05) different values. Error bars show SE values.

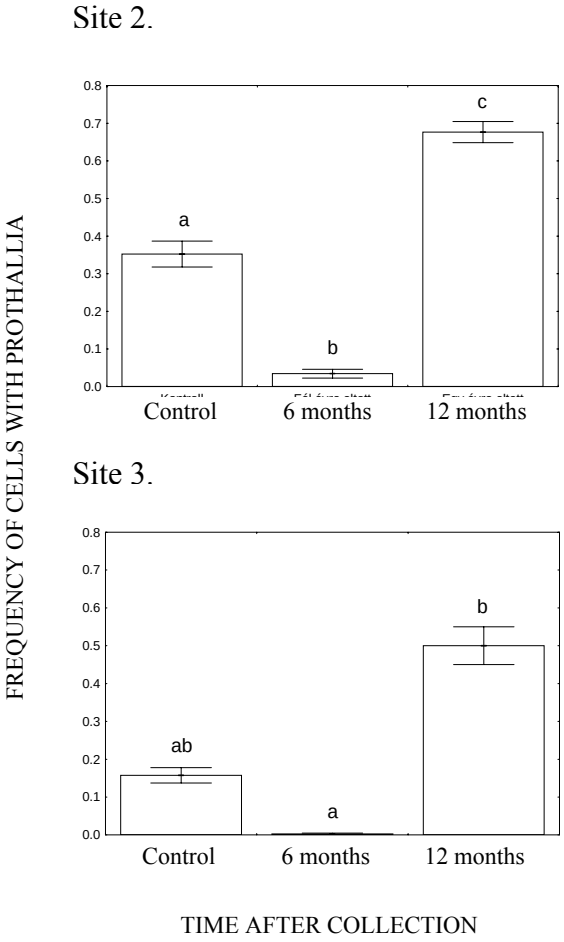


Fig. 2 Effect of storage on the frequency of prothallia emerging from the soil samples (n=50). The different letters represent significantly (P≤0.05) different values. Error bars show SE values.

Discussion

Except a few *Asplenium ruta-muraria* sporophytes growing in rock cracks, no other fern species were found at the study sites. As expected, sporophytes of this species emerged in greatest frequency from the soil samples, but another five species were also present. Several fern species may thus occur in the surrounding habitats, which explains their presence, although in lower abundance, in the soil samples. Fern spores are generally most abundant around the mother plant (Schneller 1999; Penrod and McCormick 1996) but are able to travel great distances (e.g. Ramírez-Trejo et al. 2004). Since spores of many fern species are long-lived (Lloyd and Klekowski 1970), they can persist for years in the soil if conditions at the surface are not favourable for germination. Occurrence in the spore bank of species from outlying areas is welldocumented (During and ter Horst 1983; During et al. 1987; Leck and Simpson 1987).

Most species, such as *Athyrium filix-femina*, *Cystopteris fragilis*, and *Dryopteris* species (mostly *D. filix-mas*), occur in the nearby mesophytic forests. These possible spore sources are situated within a radius of 0.5–2 km from the study sites. *Asplenium trichomanes*, found in some soil samples, is frequently found on basic substrates, including cracks of calcareous rocks in grasslands or even forests. Although it has not been found at our study sites, presence of its spores in the propagule bank indicates propagule dispersal from the surrounding hills.

Seasonal variations observed in this study probably originated from variations in the spore bank of *Asplenium ruta-muraria* being the only species present at the study sites. The closed site showed less pronounced variations than the open ones, which can be explained by its more even microclimatic conditions throughout the year. Dispersal of most fern species found in the spore bank occurs during summer months (July-September; Simon 2000). Spore release of *Asplenium ruta-muraria* is known to be drawn out due to the relatively ineffective annulus movement (Schneller 1995), however, most of the spores produced are released within a short time after ripening. These facts support the results that the greatest frequency of prothallia was detected in samples collected in summer, which is in accordance with the results of During and ter Horst (1983), who described a peak in the amount of emerging prothallia after spore dispersal. Most of the species occurring only rarely in the soil cores (e.g., *Asplenium ceterach*, *Asplenium trichomanes*, *Athyrium filix-femina*) were also found in the August samples.

Although exact abundance values could not have been assigned to each species owing to the difficulties in identifying all young plants, prothallia of *Asplenium ruta-muraria* were by

far the most abundant in the stored samples too. Besides need of light, spores of this species are known to have a high minimum temperature requirement for germination, which is supposed to impose dormancy until the summer or late spring following spore dispersal (Young 1985, Pangua et al. 1994). Field observations about spore germination of the species are sparse. Available data (Schneller pers. comm.) show, that individuals of *Asplenium ruta-muraria* in different developmental stages can be found throughout the year due to the formerly described characteristics of spore dispersal. However, most of the spores germinate in late spring, when the high temperature requirements are fulfilled (Schneller, pers. comm.).

The frequency of prothallia was relatively small in February 2002 samples compared to those from August 2001 and June 2002. Considering results of the storage experiment and the fact that most fern spores are known to survive for longer periods (Lloyd and Klekowski 1970; Smith and Robinson 1975; Herrero et al. 2002; Ramírez-Trejo et al. 2004), the loss of viability of the majority of the spores during unfavourable periods as a possible explanation (Pangua et al. 1994; Ascott and Sheffield 2000) can very probably be excluded. Aragon and Pangua (2004) compared germination percentages of *Asplenium ruta-muraria* spores sown out immediately after collection and spores stored for 1, 6 and 12 months at -20, 5 and 20ºC under wet and dry conditions. They found no germination at -20ºC, and germination percentages were initially reduced at 5ºC as well. At the study sites winter temperatures vary approximately between -15 and 10ºC and moisture conditions also fluctuate. On the basis of these data and the observed changes in the soil samples taken at different dates, it must be hypothesized, that the decrease obtained in February 2001 samples was probably due to two factors: to a lesser extent to the death of spores and to a greater extent to the development of induced dormancy in the others. In this case breaking of this presumed dormancy could have contributed to the increase in June 2002 samples compared to those from February 2001. The surprisingly increased frequency of prothallia emerging from soil samples stored for 1 year also suggests that at least some of the spores present in the soil were dormant. Similar results were obtained by Schneller (1975) from stored spores of some *Dryopteris* species, where older spores showed a significantly higher germinability than newly produced ones. Aragon and Pangua (2004) obtained a similar pattern when storing dry spores of *Asplenium ruta-muraria* at 5ºC with the difference, that germination percentages increased earlier, namely after 6 months of storage. Germination percentages of spores stored at 20ºC remained constantly low (about 30%) throughout their experiment. The different outcome of our experiment may result from the fact that Aragon and Pangua (2004) did not test the effect of other temperatures between 5 and 20ºC, where our samples were stored. It is very probable

that germination percentages would show a continuous reaction to temperature when using a more detailed gradient. Additionally, the changements in the soil (changed microbial activity, degradation of some inhibitory substances, etc.) within which our spores were stored could also have contributed to the differences between the results of the two studies.

It is hard to speculate about the type and development of the supposed dormancy due to the lack of empirical data. Since temperature and light conditions were nearly constant and favourable during the storage and cultivation of the soil samples, it is possible, that in the field unfavourable winter conditions induce dormancy in the spores (low frequency of prothallia in February) which is later broken by the higher late spring temperatures.

Effects of the seasonally varying temperature conditions on the fronds or on the buried spores in the field could, among others, also have resulted in seasonal changes in germination requirements.

Further experiments on the development, type and regulation of dormancy as well as germination experiments in the field would thus be of great interest, especially in *Asplenium* species adapted to dry habitats.

Acknowledgements

The authors are grateful to Dr. István Pintér for providing some facilities to the study and for kindly helping us in problematic questions and to Prof. Dr. Jakob Schneller for reading, correcting and commenting the manuscript. Three anonymous referees are gratefully acknowledged for their valuable comments on the manuscript.

References

Aragon CF, Pangua E 2004 Spore viability under different storage conditions in four rupicolous *Asplenium* L taxa. *Am Fern J* **94**: 28–38

Ascott CJ, Sheffield E 2000 The effect of spore density on germination and development in *Pteridium*, monitored using a novel culture technique. *Am Fern J* **90**: 91–99

Bisang I 1996 Quantitative analysis of the diaspore banks of bryophytes and ferns in cultivated fields in Switzerland. *Lindbergia* **21**: 9–20

Dobolyi K 1997 Phytosociological studies on the rocky grasslands in Csíki-hegyek (Budaörs, Hungary). *Ann Hist-Nat Mus Natl Hung* **89**: 53–62

Dobolyi K 2002 Studies of the vegetation dynamics on the rock grasslands in Csíki-hegyek (Budaörs, Hungary). *Stud Bot Hung* **33**: 83–96

Dobolyi K, Kováts D, Szerdahelyi T, Szollát Gy 1991 Vegetation studies on the rocky grasslands of Odvas Hill (Budaörs, Hungary). *Ann Hist-Nat Mus Natl Hung* **83**: 199–223

Draskovits R, Kovács-Láng E 1968 Mikroklimamessungen in Kalkstein- und Dolomitfelsenrasen. *Ann Univ Sci Budap Rolando Eötvös Nominatae, Sect Biol* **9–10**: 115–129

During HJ, Brugués M, Cros RM, Lloret F 1987 The diaspore bank of bryophytes and ferns in the soil in some contrasting habitats around Barcelona, Spain. *Lindbergia* **13** :137–149

During HJ, ter Horst B 1983 The diaspore bank of bryophytes and ferns in chalk grassland. *Lindbergia* **9**: 57–64

Dyer AF, Lindsay S 1992 Soil spore banks of temperate ferns. *Am Fern J* **82**: 89–122

Herrero A, Prada C, Pajarón S 2002 Gametophyte morphology and gametangial ontogeny of *Asplenium foreziense* and related taxa (Aspleniaceae: Pteridophyta). *Bot J Linn Soc* **139**: 87–98

Hock Zs 2003 *Dolomit sziklagyepek kriptogám diaspórabankjának vizsgálata. (Diaspore bank of bryophytes and ferns in dolomite rock grasslands).* MSc thesis, manuscript, Budapest

Leck MA, Simpson RL 1987 Spore bank of a Delaware River freshwater tidal wetland. *Bull Torrey Bot Club* **114**: 1–7

Lloyd RM, Klekowski JR 1970 Spore germination and viability in Pteridophyta: evolutionary significance of chlorophyllous spores. *Biotropica* **2**: 129–137

Pangua E, Lindsay S, Dyer AF 1994 Spore germination and gametophyte development in three species of *Asplenium*. *Ann Bot (Oxford)* **73**: 587–593

Penrod KA, McCormick LH 1996 Abundance of viable hay-scented fern spores germinated from hardwood forest soils at various distances from a source. *Am Fern J* **86**: 69–79

Raghavan V 1989 *Developmental biology of fern gametophytes.* Cambridge University Press, Cambridge

Ramírez-Trejo MdR, Pérez-Garcia B, Orozco-Segovia A 2004 Analysis of fern spore banks from the soil of three vegetation types in the central region of Mexico. *Am J Bot* **9**: 682–688

Ranal MA 2003 Soil spore banks of ferns in a gallery forest of the Ecological Station of Panga, Uberlândia, MG, Brazil. *Am Fern J* **93**: 97–115

Rydgren K, Hestmark G 1997 The soil propagule bank in a boreal old-growth spruce forest: changes with depth and relationship to above-ground vegetation. *Can J Bot* **75**: 121–128

Schneller JJ 1975 Untersuchungen an einheimischen Farnen, insbesondere der *Dryopteris filix-mas*-Gruppe (3) Teil. Ökologische Untersuchungen. *Ber Schweiz Bot Ges* **85**: 110–159

Schneller JJ 1988 Spore bank, dark germination and gender determination in *Athyrium* and *Dryopteris*. Results and implications for population biology of pteridophyta. *Bot Helv* **98**: 77–86

Schneller JJ 1995 Aspects of spore release of *Asplenium ruta-muraria* with reference to some other woodland ferns: *Athyrium filix-femina*, *Dryopteris filix-mas*, and *Polystichum aculeatum*. *Bot Helv* **105**: 187–197

Schneller JJ 1999 How much genetic variation in fern populations is stored in the spore banks? A study of *Athyrium filix-femina* (L) Roth. *Bot J Linn Soc* **127**: 195–206

Simon T 2000 *A magyarországi edényes flóra határozója.* Nemzeti Tankönyvkiadó, Budapest

Simpson K, Dyer A 1999 The survival of dormant fern spores. *Pteridologist* **3**: 98–103

Smith DL, Robinson PM 1975 The effects of spore age on germination and gametophyte development in *Polypodium vulgare* L grown under red and blue light. *New Phytol* **72**: 1261–1268

Sokal RR, Rohlf FJ 1995 *Biometry. The principles and practice of statistics in biological research.* Freeman and Company, New York

Spiess LD, Krouk MG 1977 Photocontrol of germination of spores of the fern *Polypodium vulgare*. *Bot Gaz* **138**: 428–433

SPSS Inc 1999 *SPSS Base 9.0 for Windows User's Guide.* SPSS Inc, Chicago IL

Stokey AG 1951 The contribution by gametophyte to the classification of the homosporous ferns. *Phytomorphol* **1**: 39–58

Strickler GS, Edgerton PJ 1976 Emergent seedlings from coniferous litter and soil in eastern Oregon. *Ecology* **57**: 801–807

Young JE 1985 Some effects of temperature on germination and protonemal growth in *Asplenium ruta-muraria* and *A. trichomanes* (Abstr.). *Proc R Soc Edinb* **86B**: 454–455

Zólyomi B 1942 A középdunai flóraválasztó és a dolomitjelenség. (Die Mitteldonau-Florenscheide und das Dolomitphänomen). *Bot Közl* **39**: 209–231

Zólyomi B 1958 Budapest környékének természetes növénytakarója. In: Pécsi M (ed) *Budapest természeti képe.* Akadémiai Kiadó, Budapest, pp 533–555

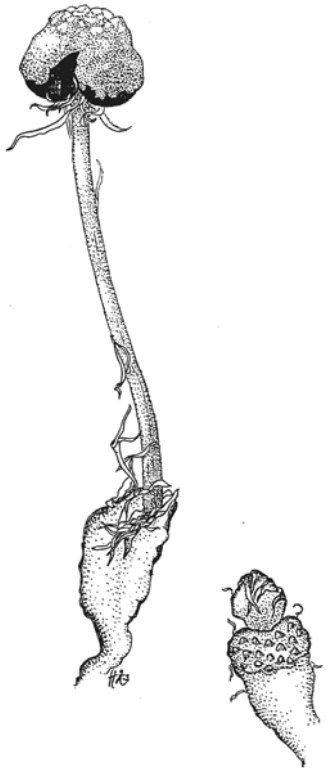
Plate 4 Fern prothallia and young plants emerging from soil samples. A. Prothallium with hairs. B, D. Naked prothallia – *Athyrium* spp. C. Young *Asplenium ruta-muraria* L. plants. E. Young, slightly deformed *Asplenium trichomanes* L.

Chapter III.

Population genetic consequences of the reproductive system in the liverwort *Mannia fragrans* (Balb.) Frye and Clark

Zsófia Hock[1,2], Péter Szövényi[1,2], Jakob J. Schneller[1], Edwin Urmi[1] and Zoltán Tóth[2]

[1]*Institute of Systematic Botany, University of Zürich, Zürich, 8008, Zollikerstr. 107, Switzerland;*
[2]*Eötvös Loránd University, Budapest, 1117, Pázmány Péter s. 1/C, Hungary*



Under review in

American Journal of Botany

Abstract

Ecological factors affecting reproduction and dispersal are particularly important in determining genetic structure of plant populations. Using the polyoicous liverwort *Mannia fragrans* as model species, we test the relative importance of the components of its unexplored reproductive system and assess consequences on the genetic structure of populations. Sex expression increased with patch size and sex ratios were female biased. Additional input into clonal growth after production of sex organs was found in males compared to females. Based on similarities in clonal traits of the rare bisexual thalli with non-expressing plants and their more frequent occurrence in small patches, we hypothesize that selection prefers colonizers that first develop organs of both sexes, ensuring sexual reproduction when no partner is present. Despite frequent spore production, ISSR markers revealed low genetic diversity, resulting from the effective clonal propagation of the species and frequent crossing between genetically identical plants. Remote populations differed significantly, each being dominated by a few clones, reflecting negligible gene flow among them. Presence of rare alleles and unique recombinant haplotypes indicates occasional recombination and mutation. Spreading of these is, however, hampered by large spore size. Since populations are small and isolated, such haplotypes are probably continuously eliminated by genetic drift.

Keywords: clonal traits; genetic diversity; liverwort; *Mannia fragrans*; polyoicous; reproductive ecology; sex expression; sex ratio

Introduction

Life-history characteristics, such as dispersal modes and their efficiency, breeding system and frequency of sexual reproduction may considerably alter the extent and partitioning of genetic variability (Loveless and Hamrick, 1984). In bryophytes, capacity for dispersal decreases with increasing propagule size (Söderström and Herben, 1997). If suitable habitats are rare and discontinuous and populations small, a species with large propagules will experience reduction of within population genetic variability, due to strong genetic drift and the lack of effective migration between populations. Within population genetic variability will be further reduced by frequent inbreeding and clonal propagation within isolated stands (Dewey, 1989; Shaw and Schneider, 1995; Boisselier-Dubayle and Bischler, 1997).

At the same time, genetic differences among isolated populations of the same species are expected to be high, with rarity of widespread clones and with unique haplotypes occurring at remote stands (Loveless and Hamrick, 1984). The same pattern is generally found in primarily clonal plants (Ellstrand and Roose, 1987), or when long-range dispersal is ineffective. In the latter case, more recently colonized sites should show lower genetic diversity, since simultaneous colonization by different haplotypes is less probable.

The distribution of genetic variability is further influenced by sex-expression rates and the relative frequencies of male and female plants, since lack of one sex or skewed sex ratios hamper or reduce the chance of fertilization. Sex expression depends on several factors including environmental parameters (Stark et al., 2001) and patch size (McLetchie and Puterbaugh, 2000). Larger patches have greater microsite diversity and presence of more potential partners may stimulate sex expression as well (Chopra and Sood, 1973). In bryophytes, sex ratios are very often skewed, with prevalence of females among unisexual species (Bisang and Hedenäs, 2005). Underlying causes range from differential germination and survival to differing environmental requirements, tolerance and clonal growth patterns of sexes (Bisang and Hedenäs, 2005).

Life history theory predicts that in case of limited resources, a negative correlation should exist between resources invested in current reproduction and future survival, growth and reproduction (Stearns, 1989). Although cost of reproduction is supposed to be relatively easy to measure in bryophytes due to their low ability to compensate it, attempts to assess it are sparse (Rydgren and Økland, 2003). Differential costs of sexuality in males and females have been found in a few cases (Stark, 2002a) and higher costs were detected in fertilized than in unfertilized females (Rydgren and Økland, 2003).

Mannia fragrans (Balb.) Frye and Clark is a thallose, xerophilous liverwort growing on bare soil in open, exposed patches of dry grasslands. The reproductive system of the species is intriguing. Despite being described as polyoicous (i.e. populations consisting of bisexual gametophytes and unisexual gametophytes of both sexes), the amount of bisexual thalli in its populations is often very low (pers. obs.), and several populations seem to lack such plants (Damsholt, 2002). As far as we can ascertain, only descriptive data mostly related to taxonomy exist about polyoecy in bryophytes (e.g. Wyatt and Anderson, 1984) and no attempts have been made to understand the role and significance of this phenomenon in population biology. In *Mannia*, both clonal propagation by fragmentation and sexual reproduction are very intensive (Damsholt, 2002). The use of genetic tools may help to separate the extent to which different reproductive modes contribute to the genetic composition of populations.

Populations of *Mannia fragrans* are geographically isolated, since suitable habitats generally occur as small islands within other vegetation types. In spite of its regular sexual reproduction, dispersal capacity of the species through spores is probably weak, due to its large spore size (60-80µm, Damsholt, 2002), which enhances isolation of populations. Isozyme studies conducted on *M. fragrans* samples from different geographical locations found low polymorphism within and among populations of the species (Odrzykoski and Szweykowski, 1981). Hence, a high percentage of fertilization may occur between genetically identical thalli, equaling clonal propagation, which may decrease genetic diversity. In addition, given the polyoicous reproductive system of the species, intra-gametophytic selfing may occur as well depending on the frequency of bisexual forms. Last but not least, very effective asexual reproduction by fragmentation of thalli in *M. fragrans* also suggests low within population genetic variability (Ellstrand and Roose, 1987). It must however, be stated, that low polymorphism obtained in the former study may be the consequence of the low resolution of isozymes. Genetic markers with higher resolution, such as ISSRs (Godwin et al., 1997), may reveal more variability.

The objectives of the present study are to assess the reproductive ecology of *Mannia fragrans* and to test to what extent reproductive characteristics influence genetic structure within and among populations. More specifically, we are interested in sex-expression rates in relation to patch size, sex-ratio patterns and cost of reproduction, assessed as sex-specific clonal traits. Genetic investigations focus on the relative success of sexual vs. clonal reproduction based on the genetic structure of the investigated populations.

Materials and methods

Study areas

Three Hungarian populations of *Mannia fragrans* with different characteristics were sampled (Fig. 1, Plate 5).

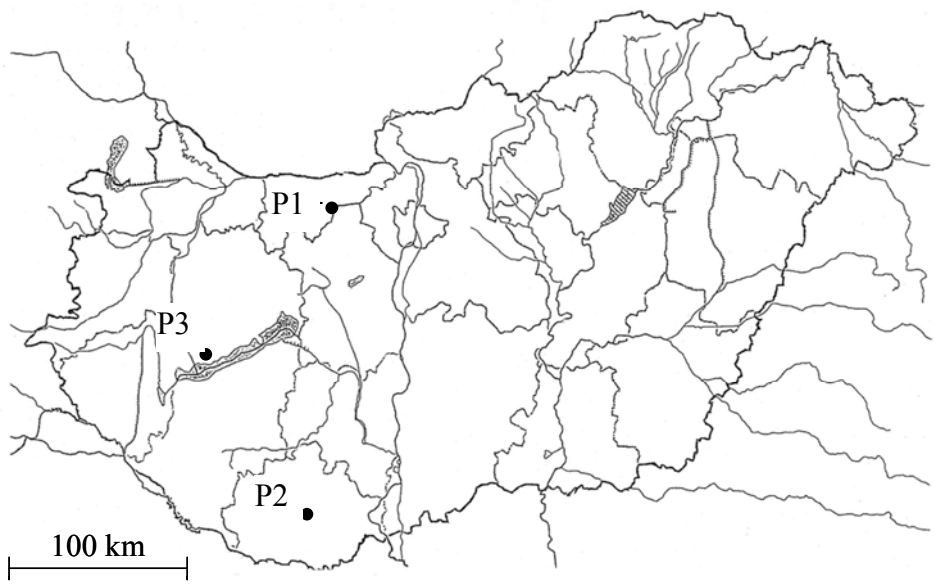


Fig. 1 Location of the three populations investigated.

A population was defined as a group of patches of the species occurring at a restricted station. Population 1 grows on rendzina covered open patches of a grassland on dolomite rock in the Vértes Mountains (N 47°31'21", E 18°29'57"). This is a small, probably mainly asexually reproducing population (pers. obs.). A series of populations of different sizes occurs on neighbouring slopes (100-1000 m away), separated from the investigated site by low-canopy forest patches. Population 2 is situated in the Mecsek Mountains (N 46°06'09", E 18°12'27"). This is a slightly bigger population growing on limestone. Population 3 grows among the basalt rocks of the southwest facing, steep slope of Szent György Hill (N 46°49'39", E 17°29'55"). The two latter populations frequently and abundantly produce sporophytes (observed since 1996). Both are more isolated than the first one. They are surrounded by forests and agricultural landscape, with the closest populations of the species being 10-30 kilometres away. Populations 1, 2 and 3 are further referred to as P1, P2 and P3, respectively.

Sampling occurred three times, in the main vegetation periods of the grasslands: (1) November 2004, before spore production, (2) April 2005, immediately after spore dispersal, (3) November 2005. Population 3 was only sampled in November 2005.

Sampling and DNA analysis

At each locality, all patches of individuals were sampled, marked, photographed and represented on a map. Number of individuals growing in each patch was noted, as well as sex ratios per patch. A sample, generally containing 5 plants/patch (depending on the size of the patch) was taken from each of them.

Collected individuals were manually cleaned under a dissecting microscope. In order to exclude potential fungal contaminants, which are reported as being common in liverwort thalli (Read et al., 2000), rhizoids and ventral scales were thorougly removed and only the green, apical parts were used in the genetic analyses. To remove small soil particles, each plant was put in de-ionized water and stirred during 5-10 minutes using a magnetic stirrer.

DNA was extracted using the Quiagen Dneasy Plant Mini Kit following the manufacturer's instructions with a modified final step because of the small amounts of plant material. In order to concentrate the samples, instead of washing and incubating the samples with AE buffer, we washed them twice with 100 µl ddH_2O. They were then centrifuged for 1 min at 8000 rpm. Water was evaporated using a DNA 120 SpeedVac vacuum concentrator and the DNA diluted with 30 µl AE buffer. For further analyses, ISSR markers were chosen because of their reliability and success in other population studies (Wolfe and Liston, 1998; Gunnarsson et al., 2005; Hassel et al., 2005). During preliminary studies only three primers yielded satisfying results, however they showed a considerable number of polymorphic loci (Table 1).

3 µl of DNA (2 ng/µl) was added to a reaction mixture containing 9.95 µl ddH_2O, 3.35 µl 25 mM $MgCl_2$, 2.5 µl 10xbuffer (100 mM Tris-HCl, pH 8.3, 500 mM KCl, 15 mM $MgCl_2$ and 0.01% gelatin), 4 µl 1.25 mM dNTPs, 2 µl primer and 0.2 µl 5u/µl Taq polymerase (Sigma). DNA was amplified on a Biometra T1 thermocycler using the following program: 4 min at 94° C and 35 cycles of 1 min at 94°C, 2 min at primer specific annealing temperatures (cf. Table 1) and 2 min at 72°C followed by a final 7-min extension at 72°C. Amplification products were visualised by agarose gel electrophoresis (1.4%). Bands were scored manually and a table of presence/absence of ISSR bands was established. PCR

Primer name	Sequence (5`-3`)	Annealing temp. (°C)	N° loci	N° polymorphic loci
UBC 834	AGAGAGAGAGAGAGAGYT	45	25	25
UBC 888	BDBCACACACACACACA	46	20	20
UBC 889	DBDACACACACACACAC	51	22	21

Y = C, T; B = C, G, T; D = A, G, T

Table 1 Primers used in the study.

reaction and/or extraction was repeated in case of problematic samples or those yielding very different patterns.

Estimation of clonal traits and testing for outcrossing

To estimate the effect of formation of sex organs on clonal traits, from all sampling dates altogether 385 thalli from P1 and P2 were cultivated for two months in moistened, closed plastic bags under ca. 1000 μmol PAR m^{-2} s^{-1}. Since most plants in cultivated samples from P3 were killed by a fungal infection, no estimations were done for this population. Prior to cultivation, the sex state of each plant was determined. After two months, further three parameters were noted for each cultivated individual: number of bifurcations and number of sub-apical and lateral branches. To test the cost of realized sexual reproduction in females, 14 female plants without sporophytes and 28 plants with mature, dehisced sporophytes from the second sampling date were cultivated in the same way. The above-mentioned parameters were then noted for these plants as well.

To gain information about sex ratios and rate of sex expression, sex state of further 1477 thalli originating from all populations and all sampling dates was established.

In order to collect information about outcrossing, non-dehisced sporophytes of 17 archegoniophores from P2 were analysed using ISSR markers along with the 14 mother plants belonging to these sporophytes.

Vegetative reproduction by fragmentation was investigated by cultivating the following cleaned 1-1.5 cm fragment types for one year on sterilized soil: 232 green fragments including apex, 206 brownish but not yet decaying fragments from below this region (some ventral-lateral primordia were often observed on such parts during microscopical cleaning of plants) and 27 apical parts of female plants with opened capsules.

Data analysis

Population sex ratios were estimated for each population separately, at the following levels: pooled values for all sampling dates, sampling dates separately and at the patch level. Since not all patches had enough samples available for statistical analysis, counts from each patch have been pooled over sampling dates. Deviation from the 1:1 sex ratios were investigated with Fisher's exact test and, in case of high number of observations, the maximum likelihood chi-square test (Sokal and Rohlf, 1995).

To test whether the sex state of the thalli was associated with clonal traits and patch size, a log-linear analysis was conducted (Sokal and Rohlf, 1995). If two traits are associated, a significant interaction between them is expected. Significance of the interaction was tested by determining the change in the log-likelihood ratio chi-square after adding or deleting the given interaction from the model. Sex state included four categories: males, females, non-expressing and bisexual plants. The number of bifurcations and number of lateral branches were divided into three (0, 1, ≥2), the number of sub-apical branches into two classes (0, ≥1). For patch sizes, two categories were established: small patches containing 1-100 individuals, large patches containing more than 100 individuals. Altogether 385 plants were analysed (93 males, 152 females, 123 non-expressing and 17 bisexual plants).

To test the effect of sporophyte production on the number of bifurcations and lateral and sub-apical branches data from 28 individuals with and 14 individuals without mature sporophytes from the second sampling date were compared using a chi-square test. We divided the different traits into the same categories as described above.

The effect of fragment type on regeneration ability was tested with a maximum likelihood chi-square test (Sokal and Rohlf, 1995). All analyses described above were conducted with the SPSS software package (SPSS for Windows 11.0.1).

Standard genetic indices including the number of polymorphic loci (S), average gene diversity over loci (H_S, Nei 1987), mean haplotype diversity (h_S, Nei 1987), occurrence of shared haplotypes were calculated for all populations at all sampling dates. The genetic structure was examined by an analysis of molecular variance (AMOVA). This method was used to partition the genotypic variance among and within populations at each sampling date. Levels of significance were determined through the computing of 1000 random permutations replicates. These analyses were performed using the ARLEQUIN 3.01 software package (Excoffier et al., 2005). The number of alleles and the number of private alleles/population

were additionally established for each sampling date using GenAlEx 6 (Peakall and Smouse, 2006).

In order to gain information about the relative importance of sexual recombination compared to that of somatic mutation in creating genetic diversity, the incompatibility excess ratio was established for all populations at all sampling dates using the PICA 4.0 software (Wilkinson, 2001). In two binary character data, such as the presence or absence of ISSR bands at two loci, the presence of all four possible combinations of characters (0/0, 1/0, 0/1, 1/1) is more parsimoniously explained by recombination than by three mutation events. This is referred to as incompatibility, and can be used as a measure of recombination when summed over all pairwise comparisons. In case of matrix incompatibility, the contribution of a particular genotype was calculated by jackkniving using the JACTAX option (using empirical frequencies and 1000 randomizations) in PICA (Wilkinson, 2001) to determine the proportion of unique genotypes that is likely the result of mutation, and thus is part of a clonal lineage.

To evaluate the association among loci in each population and to explore if allele distributions originate from sexual or asexual reproduction, we used an estimate of multilocus linkage disequilibrium independent of sample size (r_d); calculated by use of the Multilocus 1.2 software (Agapow and Burt, 2000), and 1000 artificially recombined data sets were used to determine the statistical significance of the test.

Results

Sex expression and population sex ratios

Population/ sampling	N° ♀	N° ♂	χ^2
Pop 1/2	25	2	13.251*
Pop 1/3	199	65	40.691*
Pop1/all	224	67	*46.578**
Pop 2/1	78	30	11.342*
Pop 2/2	129	39	26.431*
Pop 2/3	324	111	*56.304**
Pop 2/all	531	180	*92.317**
Pop 3/3	637	196	*127.757**

* = P<0.001

Table 2 Deviation of sex ratios from 1:1. Results from the different populations at all sampling dates. Significance values originate from the Fisher's exact test, except for high sample numbers, where the maximum likelihood $\chi 2$ test was performed (*italics*).

Sex state of thalli was significantly correlated with patch size (Table 3, Plate 6): large patches had more sex-expressing individuals (83%, n=141) than small ones (59%, n=244). Sex ratios were significantly female-biased at all sampling dates for all sites investigated (Table 2). Sex ratios of individual patches showed a similar tendency: counts of male plants were always lower than those of females in all patches in both populations. In P1, this relationship was significant in 4 of 9 patches ($P<0.05$), and marginally significant ($0.05<P<0.06$) in further one patch according to the results of the Fisher`s exact test. In P2 significant differences were found in 5 out of 14 patches ($P<0.05$), and marginally significant differences in further two patches ($0.05<P<0.07$). The frequency of bisexual plants was very low at all sites investigated (1-2%).

Sex-specific clonal traits

When comparing females and males only, no difference was found between them in the number of bifurcations and lateral branches (Table 3). However, significant association was found between sex state and the number of sub-apical branches, with higher numbers in males (Plate 7) than in females (no branches: 28%, n=93, 92%, n=152, respectively). Patch size and the number of lateral branches were also associated (Plate 5): in smaller patches less such branches were formed (no lateral branches: 72%, n=244, 78%, n=141, for small and large patches, respectively). This latter association was found when using males and females in the analysis.

When only bisexual plants were excluded from the analysis, the same association was found between sex state and number of sub-apical branches. Additionally, significant associations were obtained between sex state and number of bifurcations and number of lateral and sub-apical branches. Non-expressing plants had significantly more bifurcations

(plants with bifurcations: 45%, n=262, 82%, n=123 for expressing and non-expressing plants, respectively) and significantly less sub-apical and lateral branches than males or females (plants with lateral branches: 29%, 12%, with sub-apical branches: 34%, 6%, for expressing and non-expressing plants, respectively; Plate 6).

Association tested with sex	Model	df	Pearson $\chi 2$
N° bifurcations	LB, AB, PB, AL, PL, PA, S	121	347.73
(SB)	LB, AB, PB, AL, PL, PA, BS	115	273.59
	BS	6	74*
N° lateral branches	LB, AB, PB, AL, PL, PA, S	121	347.73
(SL)	LB, AB, PB, AL, PL, PA, LS	115	302.39
	LS	6	45*
N° apical branches	LB, AB, PB, AL, PL, PA, S	121	347.73
(SA)	LB, AB, PB, AL, PL, PA, AS	118	171.84
	AS	3	176*
Patch size	LB, AB, PB, AL, PL, PA, S	121	347.73
(SP)	LB, AB, PB, AL, PL, PA, PS	118	333.83
	PS	3	14*
N° bifurcations	LB, AB, PB, AL, PL, PA, SL, SA, SP	109	138.36
within sex (SB)	LB, AB, PB, AL, PL, PA, SL, SA, SP, SB	103	86.563
	SB	6	52*
N° lateral branches	LB, AB, PB, AL, PL, PA, SB, SA, SP	109	106.7
within sex (SL)	LB, AB, PB, AL, PL, PA, SA, SP, SB, SL	103	86.563
	SL	6	20*
N° apical branches	LB, AB, PB, AL, PL, PA, SB, SL, SP	106	210.58
within sex (SA)	LB, AB, PB, AL, PL, PA, SP, SB, SL, SA	103	86.563
	SA	3	124*
Patch size	LB, AB, PB, AL, PL, PA, SB, SL, SA	106	99.3
within sex (SP)	LB, AB, PB, AL, PL, PA, SB, SL, SA, SP	103	86.563
	SP		13*

Table 3 Log-linear analysis of associations between the number of bifurcations, of lateral and sub-apical branches and patch size and the sex state of *Mannia fragrans*. Statistical significance of each interaction was tested by determining the change in the log-likelihood ratio after adding or deleting that specific interaction from the model. S=sex state (1, 2, 3, 4), B=n° bifurcations, L=n° lateral branches, A=n° sub-apical branches, P=patch size. *P<0.05. None of the three or higher order interactions was significant. When excluding bisexual or bisexual and sterile plants from the analysis, the following interactions were found to be significant (P<0.05): SB, SA, SP, AL and B, PL, SA, respectively.

Finally, analyses of all sex states, including bisexual plants as well, yielded an additional association between sex state and number of lateral branches. Compared to other sex states, the number of plants producing two or more lateral branches was highest in bisexual plants (47%, n=17, 10% n=368 for bisexual plants and other sex states together, respectively; Plate 6). Similarly, the number of bifurcations was also higher in bisexuals (76%, n=17) than in other sex states (56%, n=368).

Significant differences between females with and without sporophytes were only found in the number of sub-apical branches, which was proportionally higher in the latter ($\chi 2$ = 10.18, df = 2, P<0.001).

Importance of sexual vs. clonal reproduction

From the cultivated fragments, green parts including the apex showed significantly higher regeneration capacity than brownish lower ones (χ^2=55.658, df=1, P<0.05). Fragments of female plants with sporophytes, having shed spores immediately before sampling, showed no regeneration at all (Plate 8).

Of the 17 archegoniophores analysed, 10 were genetically identical to the mother plant, the remaining 7 differed. An example of the latter is shown in Table 4.

♂	0	0	***1***	1	***1***	0	1	0	1	0	1	0
♀	1	0	0	1	0	1	1	1	1	0	1	0
Aph	1	**1**	***1***	1	***1***	1	1	1	1	**1**	1	**1**

Table 4 Results of the simultaneous genetic analysis of archegoniophores and mother plants. Example of plants originating from patch 3. Aph=archegoniophore, ***1***=fragment found in male plants from the same patch, **1**=fragment found in male plants from other patches.

As an archegoniophore bears several sporophytes, genetic patterns differing from that of the mother plant may reflect fertilization of some archegonia by a genetically different male plant. Estimation of multilocus linkage disequilibrium (r_d) showed significant deviation from the hypothesis of free recombination (Table 5). Significant matrix incompatibility was only found in P1 and 2 at sampling dates 3 and 1, respectively (Table 5). Entering the samples from these two populations in the JACTAX algorithm, the number of genotypes accounting for the incompatibility and hence very likely derived from recombination was very low for both populations (4 and 2 for P1 and 2 respectively).

Population/ sampling	N°s/n°h	S	%p	N°bands/ n°private bands	N° bands under 5% freq.	N°private haplotypes	N° haplotypes under 5% freq.	H_S±SD	h_S±SD	IER	r_d
Pop1/1	43/12	50	79.4	55/19	31	6	6	0.075±0.042	0.147±0.097	0.251*	0.554*
Pop1/2	34/13	35	66.0	43/15	0	6	9	0.099±0.055	0.139±0.092	0.663*	0.692*
Pop1/3	44/13	35	76.1	44/16	18	7	9	0.074±0.043	0.136±0.089	0.081	0.454*
Pop2/1	45/13	37	58.7	45/8	20	7	7	0.055±0.032	0.126±0.084	0.005	0.450*
Pop2/2	44/13	24	45.3	38/10	14	9	9	0.049±0.030	0.126±0.084	0.603*	0.119*
Pop2/3	59/10	9	19.6	28/2	3	6	6	0.040±0.026	0.157±0.105	0.639*	0.105*
Pop3/3	41/5	7	15.2	27/0	3	2	2	0.021±0.016	0.218±0.162	1.000*	0.178*

Table 5 Genetic variability in different populations of *Mannia fragrans*. N°s/N°h = number of samples/number of haplotypes; S = number of polymorphic loci; %p = percentage of polymorphic loci; H_S = average gene diversity over loci; h_S = average haplotype diversity; IER = incompatibility excess ratio; rd = multilocus linkage disequilibrium; * = P<0.005.

Genetic diversity within and among populations

According to the AMOVA, the within-population components of variance dominated, accounting for 67-78% of the variation, with only 22-33% representing variation among populations (Table 6).

	df	Variance component	Variance (%)	Fixation index
Among populations/1	1	0.560	21.61	F_{ST} = 0.216*
Within populations/1	86	2.032	78.39	
Total/1	87	2.592		
Among populations/2	1	0.557	22.85	F_{ST} = 0.229*
Within populations/2	76	1.880	77.15	
Total/2	77	2.437		
Among populations/3	2	0.510	33.102	F_{ST} = 0.331*
Within populations/3	141	1.030	66.898	
Total/3	143	1.540		

Table 6 Results of the AMOVA. * P<0.001. 1,2,3 = sampling dates.

However, populations were significantly differentiated from each other. P1 and P2 shared some of the more frequent haplotypes, but the two dominant haplotypes in P2 were not present in P1. The two populations had a high number of rare, population-specific haplotypes as well. Such haplotypes were almost lacking in P3, which was very close to P2 when looking at the dominant haplotypes. P1 and P3 only shared one haplotype (Table 7).

Haplotype name	P1	P2	P3
A	10.74	3.38	7.32
B	19.01	10.81	-
C	14.05	0.68	-
D	11.57	0.68	-
E	9.09	10.14	-
F	4.96	5.41	-
G	0.83	0.68	-
H	-	38.51	24.39
I	-	18.24	63.41
J	10.74	-	-
K	5.79	-	-
L	3.31	-	-
M	1.65	-	-
N	-	2.03	-
% unique, rare haplotypes	*8.27*	*9.46*	*4.89*

Table 7 Haplotypic composition of the three populations investigated. Data given in percentages pooled for all sampling dates. Unique, rare haplotypes = haplotypes restricted to one of the three populations, represented by only one individual.

The genetic characteristics of the studied populations are summarized in Table 5. The number of genets compared to the number of ramets sampled was relatively low, haplotype numbers were similar in P1 and P2 (mean: 12.7, 12.0, respectively), but P3 had fewer haplotypes. A similar relationship was found in the case of the number of rare and private haplotypes. The mean percentage of polymorphic loci was highest in P1 and decreased towards P3 (74, 41, 15 %, respectively). The mean number of bands and the mean number of private bands showed similar tendencies (47.3/16.7, 37.0/6.7, 27.0/0.0 for the three populations, respectively). The mean number of rare alleles was also highest in P1 and decreased towards P3 (16.3, 12.3, 3.0, respectively). Average gene diversity also decreased towards P3 (means: 0.083, 0.048 and 0.021, respectively) but haplotypic diversity was highest in P3 and yielded similar values for P1 and 2 (0.218, 0.141, 0. 218, respectively).

Discussion

Importance of sexual vs. clonal reproduction

Sex expression – Compared to existing data about bryophytes, the overall rate of sex expression observed in this study (0.71) is relatively high, but falls within the range of values obtained for other species (Bisang and Hedenäs, 2005).

Several explanations are plausible for the higher rates of sex expression in larger patches obtained. First, large patches probably represent older colonies based on the several layers of dead plants found below them, which are lacking in small patches (Plate 6). Hence, higher rates of sex expression could simply be related to the longer time period available for reaching maturity. Second, there may also be a need to reach a treshold size prior to sex expression as well (Wiklund and Rydin, 2004). In small patches, probably representing an early colonization stage, space limitation is lacking, hence germinating plants first invest into growth. Time needed to build larger thalli with at least one bifurcation is in the range of several months (Hock, pers. obs.). Additionally, as long as enough space is available, plants continue to grow and to branch (Damsholt, 2002 and high number of bifurcations found in non-expressing plants from smaller patches). Third, sex-expression may also be stimulated by the contact with other plants. In *Mannia*, crowding is suggested to put an end to dichotomous branching and to induce production of intercalary branches (Damsholt, 2002), which usually develop sex organs later on. Whether this is induced by some chemicals as in ferns and some bryophytes (Fernandez et al., 1997, Chopra and Sood, 1973) needs further investigation. Finally, a positive influence of hydration on sex expression may be possible as well (Kumra and Chopra, 1983) through the better retention of water in large patches due to the densely packed, concave thalli, and the vaste rhizoid net below them.

Gametangial induction, probably the result of the complex interaction of numerous factors, is a poorly studied field in bryophytes: underlying causes need to be explored and existing hypotheses to be confirmed by empirical data.

Sex ratio and sex-specific clonal traits – As in the majority of dioicous bryophytes (Bisang and Hedenäs, 2005), sex ratios were strongly female biased in *Mannia fragrans*. The obtained F:M=3:1 ratio is within the range reported for other dioicous species with female predominance (Bisang and Hedenäs 2005). Male plants invested more in clonal growth: contrarily to females, where sex organs were produced on lateral branches, male organs usually terminated leading branches and lateral branches of males were generally sterile.

Males also produced considerably more sub-apical branches, often developing new antheridia in the following season. While production of antheridia in *Mannia* does not involve the apical cell, allowing continuous growth (Haupt, 1921), archegoniophores arise from the apical cell, hence stop the growth of the apex (Leitgeb, 1881). Further sub-apical growth following sporophyte production is rather exceptional, as proved by the low percentages of sub-apical branches obtained and the lacking regeneration from apical fragments in female plants with mature gametophores. It mainly occurs in case of sporophyte abortion, and involves the formation of a new growing point (Haupt, 1929 and pers. obs.; Plate 9). A differential cost of producing organs of the two sexes may explain the differences in subsequent clonal growth between them. Experimental approaches of differences in the cost of realized sex expression between sexes are sparse and do not allow to formulate generalizations (Bisang and Hedenäs, 2005). However, it can be postulated that in fertilized individuals of *Mannia*, the production of stalked female gametophores, and the large spores may need more energy than that of sessile antheridia.

But if costs of sexual reproduction are higher in females, why not more males? Without further experiments, it is hard to answer this question. It was put forward that skewed sex ratios can not generally be explained by the "cost of sex hypothesis" as proposed before (Bisang and Hedenäs, 2005). In *Mannia*, sex-specific survival or tolerance (McLetchie and Puterbaugh, 2000; Stark et al., 2001) can be excluded, since the two sexes grow intermingled. Regulation by chemical factors may be plausible (Bhatla and Chopra, 1981), however it seems more likely that sex expression is labile and dependent on seasons, age of plants or other environmental factors (Wyatt and Anderson, 1984; Korpelainen,1998). It is also likely that the production of male and female organs is sequential, as suggested in the Polytrichaceae (Glime, 2006). Since the amount of sperm cells produced by males is higher than that of egg cells, it may be possible that sex ratios are balanced at the gamete level and thus frequency dependent selection eliminates the surplus of males (Fisher, 1930).

If resources are limited, a fertilized female is expected to have fewer resources for clonal reproduction than an unfertilized one (Stark et al., 2001). In the present study, higher number of sub-apical branches in females with no or aborted sporophytes may support this hypothesis. However, it is hard to differentiate between the effect of apical dominance on the production of sub-apical branches from a potential cost of reproduction (Stark, 2002b).

In the populations investigated, bisexual thalli represented less than 1% of all plants. Analogous results were found in *Preissia quadrata*, having similar life-history traits (Haupt, 1926). Rarity of bisexual plants raises several intriguing questions that may need further

investigation. Why are such individuals so rare? What is the advantage of maintaining them in the populations and how are they maintained? Which factors lead to the expression of both sexes on the same plant? Is it possible to draw a parallel between this phenomenon and the processes observed in plants with mixed mating systems? To date, little is known about sex determination in bryophytes (Ramsay and Berrie, 1982). Monoecy is supposed to be associated with diploidy or polyploidy of gametophytes, whereas dioecy with a haploid chromosomal set (Wyatt, 1994), provided that sex determination is under genetic control. However, this is not always the case, as environmental factors or plant condition may also influence it (Korpelainen, 1998), which may be the case in *Mannia* as well. The fact that bisexual plants, similarly to non-expressing ones mostly occurred in smaller, probably newly colonized patches (pers. obs.) and that their clonal traits and size were also very close to each other (Plate 6) suggests that selection may prefer colonizers that develop sexual organs of both sexes, hence ensuring sexual reproduction when potential partners are not present. Since the species is reported to be polyoecious or unisexual (Damsholt, 2002), it would be worth investigating whether the ratio of bisexual plants varies with age of populations or geography, to elucidate the role of these individuals in the evolution of populations.

Clonal propagation by fragmentation is very effective in *Mannia fragrans*. Cultivation experiments show that apical fractions of thalli containing meristematic tips are most likely to survive periods of unfavourable conditions. New branches mainly arose from the lateral ventral region of the thalli. Similar patterns were found in a closely related species, *Asterella californica* living in areas with comparably arid summers (Haupt, 1929).

Reproductive characteristics and their footprints in population genetics

General trends in the partitioning of genetic diversity differ between the two major groups of bryophytes. While mosses usually show overall intraspecific genetic diversity levels comparable to that of vascular plants, this level is usually relatively low in liverworts (Wyatt, 1994). Underlying factors, including wider ecological amplitude of mosses, and reduced capacity for sexual reproduction of liverworts (Khanna, 1964), have been debated but are still not fully understood. In liverworts, diversity is mostly expected to be partitioned among, rather than within populations (Bischler and Boisselier-Dubayle, 1997), due to restricted dispersal ability and the relative importance of asexual reproduction. In *Mannia*, genetic composition of the three populations investigated was, indeed, different. However, most of the genetic variation was found within and not among them, which partly contradicts

observations in species having similar dispersibility, reproductive characteristics and levels of habitat specificity as *Mannia fragrans* (Szweykowski and Zielinski, 1983; Shaw and Steiner, 1995; Boisselier-Dubayle and Bischler, 1997). Average gene diversity in populations of the species was lower than that in vascular plants and in the majority of mosses but falls into the range described for liverworts and very close to that of species sharing reproductive characteristics with *Mannia* (Szweykowski and Zielinski, 1983; Boisselier-Dubayle and Bischler, 1997).

A unisexual, outbreeding species is expected to show greater levels of genetic diversity than a bisexual, inbreeding one (Loveless and Hamrick, 1984). Similar life-history characteristics and habitat preferences may predict similar genetic patterns, but this is not always necessarily so (Wyatt et al., 1989; Dewey, 1989; Stenøien and Såstad, 2001). Although the majority of liverwort species is unisexual (Wyatt and Anderson 1984), studies support little or no genetic variation (e.g. Dewey 1989, Bischler and Boisselier-Dubayle, 1993). The level of genetic variation in the predominantly unisexual *Mannia fragrans* was low as well. Low levels of genetic variation in *Preissia quadrata*, a species with similar spore size and reproductive characteristics (Boisselier-Dubayle and Bischler, 1997) were attributed to the prevalence of clonal reproduction. Fertilization and subsequent recombination provide a possibility for creating new genetic combinations. Yet, intra-gametophytic selfing and crossing between genetically identical clones result in spores genetically equivalent to asexual propagules (Wyatt et al., 1989). The rarity of bisexual plants in the populations suggest that the role of intra-gametophytic selfing is negligible. If individual patches are not genetically uniform, outcrossing between different genotypes may be expected, as indicated by our results despite of the relatively low number of archegoniophores and mother plants analysed genetically. Though, given that only a few haplotypes, very often differing in one mutation only, dominate the populations, the number of possible combinations among them is restricted; hence recombination does not necessarily lead to an increase in new haplotypes. Spreading of rare new haplotypes is hampered by large spores, mostly falling into the own patch, where there is little possibility for germination due to the densely packed thalli. Isolation and relatively small size of populations increase the effect of genetic drift on rare haplotypes. The above-mentioned hypotheses are consistent with the obtained r_d values showing very high linkage among loci compared to other species (Hassel et al., 2005; Gunnarsson et al., 2005). This suggests rare recombination events and dominant asexual reproduction within the populations in spite of the frequent sporophyte formation (83% of sex-expressing females built sporophytes).

Differences among populations: the role of reproductive characteristics

Significant differentiation of remote localities based on haplotype frequencies results from differential presence of numerous rare and some dominant haplotypes and is in line with the low dispersal ability of the species. As rare haplotypes are subject to high drift for reasons discussed above, they probably represent variation on a shorter time scale.

When looking at the alleles, the low amount of private alleles needs some further explanation. If such alleles are the results of somatic mutations, these events are probably not very frequent and the resulting new alleles are eliminated by random genetic drift. The presence of population-specific haplotypes and alleles confirms restricted gene flow hypothesized among populations. It is, however, difficult to exactly explain the occurrence of shared haplotypes. They might indicate that historical colonization of the sites occurred by a restricted number of clones only, but might also be the result of convergent evolution. As ISSR markers are not stable on a longer time scale, it is difficult to tell about historical background of the observed pattern.

The fact that P1 mostly had small patches with higher rates of non-expressing plants (30%, compared to 9 and 7% in P2 and P3, respectively) and almost no sporophytes lead to the hypothesis of this population being a mainly asexually reproducing one. Surprisingly, many of the genetic parameters (% of polymorphic loci, mean number of bands including private bands, mean number of rare alleles) were highest in this population. Matrix incompatibility at the third sampling date could suggest that recombination plays an important role in this population. This is, however, not very plausible, given the rarity of sexual reproduction. Jackkniving revealed that only 4 genotypes containing high amounts of rare alleles accounted for the obtained incompatibility, hence the majority of the plants were probably derived from the same clonal lineage by mutation. Where do then the additional haplotypes come from? Thoroughly screening the neighbourhood of the supposed "population", we found out, that in fact it represents the edge of a more extended one, composed of large patches regularly producing sporophytes. This was formerly overseen, probably because of the dry weather which rendered finding of enrolled thalli almost impossible. The fact that the sampled part of the population probably represents the expanding edge of a larger one may explain smaller patch size and less sex-expression, as well as the seemingly contradictory high levels of genetic variation found there. Additional genotypes may hence originate from mutations and recombinations in the older, larger

population through the long-lived spores (Hock, pers. obs.) waiting in the soil for new microhabitats to be formed. The fact that P1 is the only population among the investigated ones, where the closest populations of the species can be found within a kilometre may also contribute to the relatively high levels of genetic diversity found.

P2 regularly and abundantly produces sporophytes and, on the basis of population and patch size and number of individuals per patch, it was hypothesized that it represents an older colony than P1. Estimated parameters of genetic diversity were accordingly high, however, to our surprise, not higher as in P1. Possible reasons underlying this difference are discussed above.

The relative genetic homogeneity in P3 compared to the former two populations is more surprising, given its large size and similar sexual behaviour to P2. This can not simply be explained by reproductive characteristics of the species. Historical events, such as (re)colonization events, may account for it, but more extended sampling is needed to explore the background of it. Similarities with P2 may suggest recent gene flow between the two sites.

Colonization of new, remote sites is probably a rare event in *Mannia fragrans* due to its considerable spore size. For this reason, populations are composed of a few clones only. Even if sporophytes are produced in great amounts every year, a great part of fertilization takes place between genetically identical plants, producing no new genetic variation. Presence of rare alleles and numerous rare recombinant haplotypes shows that in some cases recombination and mutation give rise to new haplotypes, increasing within-population diversity. These have, however, little chance to spread in and between populations, given that most spores fall into the own patch. As populations are rather small and isolated, these rare haplotypes are probably continuously eliminated by genetic drift, though some of them may be conserved in the diaspore bank (Hock et al., in prep.).

Acknowledgements

This research was financially supported by a grant of the Hungarian Scientific Research Fund (OTKA-T047156).

References

Agapow PM, Burt A 2000 MultiLocus 1.2. *Analysing multi-locus population genetic data.* Department of Biology, Imperial College, Ascot, Berks, UK

Bhatla SC, Chopra RN 1981 Hormonal regulation of gametangial formation in the moss *Bryum argenteum* Hedw. *J Exp Bot* **32**: 1243-1256

Bisang I, Hedenäs L 2005 Sex ratio patterns in dioicous bryophytes re-visited. *J Bryol* **7**: 207-219

Bischler H, Boisselier-Dubayle MC 1993 Variation in a polyploid, dioicous liverwort, *Marchantia globosa*. *Am J Bot* **80**: 953-958

Bischler H, Boisselier-Dubayle MC 1997 Population genetics and variation in liverworts. *Adv Bryol* **6**: 1-34

Boisselier-Dubayle MC, Bischler H 1997 Enzyme polymorphism in *Preissia quadrata* (Hepaticae, Marchantiaceae). *Plant Syst Evol* **205**: 73-84

Brown AHD, Feldman MW, Nevo E 1980 Multilocus structure of natural populations of *Hordeum spontaneum*. *Genetics* **96**: 523-536

Cronberg N, Andersson K, Wyatt R 2003 Clonal distribution, fertility and sex ratios of the moss *Plagiomnium affine* (Bland.) T. Kop. in forests of contrasting age. *J Bryol* **25**: 155-162

Damsholt K 2002 *Illustrated flora of nordic liverworts and hornworts.* Nordic Bryological Society, Lund

Dewey RM 1989 Genetic variation in the liverwort *Riccia dictyospora* (Ricciaceae, Hepaticopsida). *Syst Bot* **14**: 155-167

Ellstrand NC, Roose ML 1987 Patterns of genotypic diversity in clonal plant species. *Am J Bot* **74**: 123-131

Excoffier L, Laval G, Schneider S 2005 Arlequin (version 3.0): An integrated software package for population genetics data analysis. *Evol Bioinf Online* **1**: 47-50

Fernandez H, Bertrand AM, Feito I, Sanchez-Tames R 1997 Gametophyte culture *in vitro* and atheridiogen activity in *Blechnum spicant*. *Plant Cell, Tiss Org Cult* **50**: 71-74

Fisher RA 1930 *The genetic theory of natural selection.* Oxford Univ. Press

Glime JM 2006 *Bryophyte Ecology. Vol. 1. Physiological Ecology.* http://www.bryoecol.mtu.edu/

Godwin ID, Aitken EA, Smith LW 1997 Application of inter simple sequence repeat (ISSR) markers to plant genetics. *Electroph* **18**: 1524-1528

Gunnarsson U, Hassel K, Söderström L 2005 Genetic structure of the endangered peat moss *Sphagnum angermanicum* in Sweden: a result of historic or contemporary processes? *Bryologist* **108**: 194-203

Hassel K, Såstad SM, Gunnarsson U, Söderström L 2005 Genetic variation and structure in the expanding moss *Pogonatum dentatum* (Polytrichaceae) in its area of origin and in a recently colonized area. *Am J Bot* **92**: 1684-1690

Haupt AW 1921 Gametophyte and sex organs of *Reboulia hemisphaerica*. *Bot Gaz* **71**: 61-74

Haupt AW 1926 Morphology of *Preissia quadrata*. *Bot Gaz* **82**: 30-54

Haupt AW 1929 Studies in californian Hepaticae. I. *Asterella californica*. *Bot Gaz* **87**: 302-319

Khanna KR 1964 Differential evolutionary activity in bryophytes. *Evol* **18**: 652-670

Korpelainen H 1998 Labile sex expression in plants. *Biol Rev* **73**: 157-180

Kumra PK, Chopra RN 1983 Effect of some physical factors on growth and gametangial induction in male clones of three mosses grown in vitro. *Bot Gaz* **144**: 533-539

Leitgeb H 1881 *Untersuchungen über die Lebermoose. 6. Die Marchantieen.* Graz, Austria

Longton RE, Greene SW 1979 Experimental studies of growth and reproduction in the moss *Pleurozium schreberi* (Brid.) Mitt. *J Bryol* **10**: 321-338

Loveless MD, Hamrick JL 1984 Ecological determinants of genetic structure in plant populations. *Ann Rev Ecol Syst* **15**: 65-95

McLetchie DN 1992 Sex ratio from germination through maturity and its reproductive consequences in the liverwort *Sphaerocarpos texanus*. *Oecol* **92**: 273-278

McLetchie DN, Puterbaugh MN 2000 Population sex ratios, sex-specific clonal traits and tradeoffs among these traits in the liverwort *Marchantia inflexa*. *Oikos* **90**: 227-237

Miller MP 1997 *Tools for population genetic analyses (TFPGA) 1.3. A windows program for the analysis of allozyme and molecular population genetic data.* Computer software distributed by author

Mishler BD, Oliver MJ 1991 Gametophyte phenology of *Tortula ruralis*, a desiccation-tolerant moss, in the Organ Mountains of southern New Mexico. *Bryologist* **94**: 143-153

Newton ME 1972 Sex ratio differences in *Mnium hornum* Hedw. and *M. undulatum* Sw. in relation to spore germination and vegetative regeneration. *Ann Bot* **36**: 163-178

Odrzykoski I, Szweykowski J 1981 An interesting enzymatic polymorphism in some European populations of the liverwort *Mannia fragrans* (Balbis) Frye and Clark. In: Szweykowski J (ed) *New perspectives in bryotaxonomy and bryogeography.* Adam Mickiewicz University, Poznan, Poland

Peakall R, Smouse PE 2006 GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. *Mol Ecol Notes* **6**: 288-295

Ramsay HP, Berrie GK 1982 Sex determination in bryophytes. *J Hattori Bot Lab* **52**: 255-274

Raymond ML, Rousset F 1995 An exact test for population differentiation. *Evol* **49**: 1280-1283

Read DJ, Duckett JG, Francis R, Ligrone R, Russell A 2000 Symbiotic fungal associations in 'lower' land plants. *Phil Trans Royal Soc B: Biol Sci* **355**: 815-831

Rydgren K, Økland RH 2003 Short-term costs of sexual reproduction in the clonal moss *Hylocomium splendens*. *Bryologist* **106**: 212-220

Söderström L, Herben T 1997 Dynamics of bryophyte metapopulations. *Adv Bryol* **6**: 205-240

Sokal RR, Rohlf FJ 1995 *Biometry, 3rd edn.* WH Freeman&Co. New York, NY

SPSS for Windows, Release 11.0.1. 2001 Chicago: SPSS Inc.

Stark LR 2002a Skipped reproductive cycles and extensive sporophyte abortion in the desert moss *Tortula inermis* correspond to unusual rainfall patterns. *Can J Bot* **80**: 533-542

Stark LR 2002b Phenology and ist repercussions on the reproductive ecology of mosses. *Bryologist* **105**: 204-218

Stark LR, McLetchie DN, Mishler BD 2001 Sex expression and sex dimorphism in sporophytic populations of the desert moss *Syntrichia caninervis*. *Plant Ecol* **157**: 183-196

Stearns SC (1989) Trade-offs in life-history evolution. Funct Ecol 3: 259-268

Stenøien HK, Såstad SM 2001 Genetic variability in bryophytes: does mating system really matter? *J Bryol* **23**: 313-318

Szweykowski J, Zielinski R 1983 Isoenzymatic variation in polish populations of the moss *Plagiothecium undulatum* (Hedw.) B. S. G. A preliminary report. *J Hattori Bot Lab* **54**: 119-123

Wiklund K, Rydin H 2004 Colony expansion of *Neckera pennata*: Modelled growth rate and effect of microhabitat, competition and precipitation. *Bryologist* **107**: 293-301

Wilkinson M 2001 *PICA 4.0: software and documentation.* Department of Zoology, The Natural History Museum, London

Wolfe AD, Liston A 1998 Contributions of the polymerase chain reaction to plant systematics. In: Soltis DE, Soltis PS, Doyle JJ (eds) *Molecular Systematics of Plants II: DNA Sequencing.* Kluwer, New York, pp 43-86

Wyatt R 1994 Population genetics of bryophytes in relation to their reproductive biology. *J Hattori Bot Lab* **76**: 147-157

Wyatt R, Anderson LE 1984 Breeding systems of bryophytes. In: Dyer AF, Duckett JG (eds) *The experimental biology of bryophytes.* Academic Press, London, pp 39-64

Wyatt R, Stoneburner A, Odrzykoski I 1989 Bryophyte isozymes: Systematic and evolutionary implications. In: Soltis, DE, Soltis PS (eds) *Plant Isozymes.* Dioscorides Press, Portland, OR

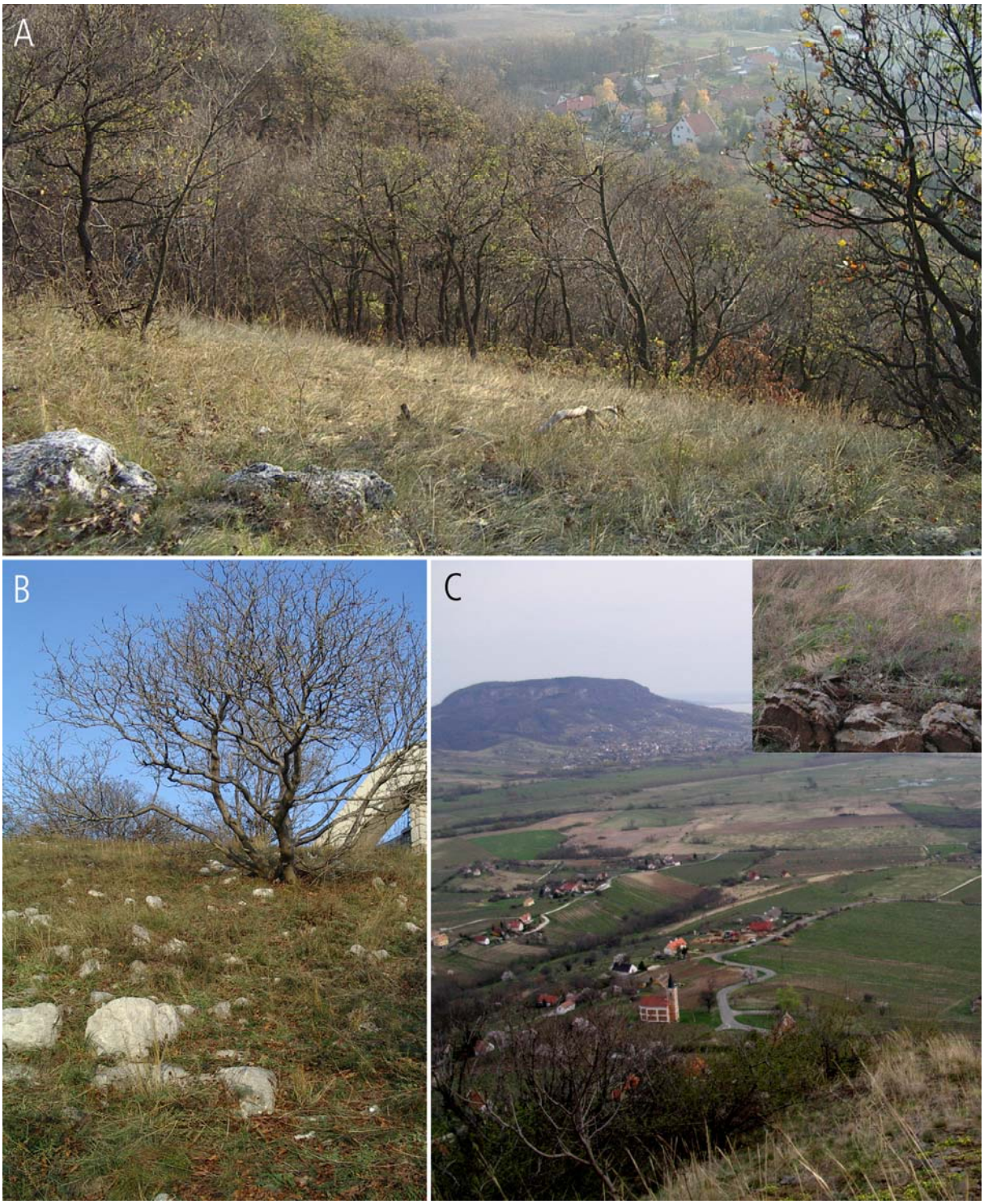


Plate 5 Sites. A. Site/Population 1 in the Vértes Mountains, dolomite bedrock. B. Site/Population 2 in the Mecsek Mountains, limestone bedrock. C. Site 3 on Szent György Hill, isolated, siliceous bedrock (small picture).

Plate 6 *Mannia fragrans* – colonization. A. Large non-expressing plant with numerous bifurcations. B. Large bisexual plant with numerous bifurcations. Its size and shape is very similar to that of non-expressing plants. C. Patch with relatively few thalli. Large non-expressing plants can be observed at the right end of the patch (arrow), where free space is available. These plants have several bifurcations but no lateral branches. D. Larger, crowded patch, probably older colony. Almost all plants express sex: lateral branches, usually bearing archegoniophores are frequent.

Plate 7 *Mannia fragrans* – male plants. A. Immature antheridia on young male plants. B. Male plant bearing antheridia from the same and the former year (a1, a2, respectively). Gametes have already been released and sub-apical innovations are being formed (I). C-D. Male plants bearing antheridia from the same year having already released gametes (a1). Sub-apical innovations are being formed (I).

Plate 8 Clonal propagation and regeneration of *Mannia fragrans*. A-B. Clonal propagation by fragmentation of thalli, plants from less crowded, smaller, probably newly colonized patches. C. Regeneration from old, distal fragments in the greenhouse. New branches arise from ventral lateral primordia. D-E. Female plants having already shed their spores usually do not regenerate. Arrows show the place of origin of archegoniophores. F. Female plant with an archegoniophore containing aborted sporophytes (Ab). A sub-apical innovation is being formed (I).

Plate 9 *Mannia fragrans* – female plants. A-B. Young, unfertilized female plants. The brush of hyaline scales from below the convex apex is protecting young archegonia and is typical for this stage. C. Female with two archegoniophores at the end of bifurcations of the main shoot. One of the archegoniophores has aborted sporophytes (Ab). Elongation of the receptacle stalk only take place at spore maturation, stalks of archegoniophores with aborted sporophytes do not elongate. D-E. Female plants from larger patches with numerous thalli. Archegoniophores are produced on ventral lateral branches of older thalli. F. Female plant bearing archegoniophores at the end of bifurcations of the main shoot. The shortness of stalks indicates the immature state of spores.

Chapter IV.

Bryophyte diaspore bank – a genetic memory? Genetic structure and genetic diversity of surface populations and diaspore bank in the liverwort *Mannia fragrans* (Balb.) Frye and Clark

Zsófia Hock[1,2], Péter Szövényi[1,2], Jakob J. Schneller[1] and Zoltán Tóth[2]

[1]*Institute of Systematic Botany, University of Zürich, Zürich, 8008, Zollikerstrasse 107, Switzerland;*

[2]*Eötvös Loránd University, Budapest, 1117, Pázmány Péter s. 1/C, Hungary*

Manuscript in preparation

Abstract

Propagule banks are essential in the life of plant populations. They help to bridge unfavourable periods and allow populations to recover after disturbance. Recent studies in vascular plants have demonstrated the role of propagule banks in influencing genetic structure and evolutionary potential of surface populations. Using *Mannia fragrans*, a model species with probably long-lived spores and a large diaspore bank specialized on temporarily available (micro-)habitats, we aimed to test whether the diaspore bank of bryophytes may also function as a "genetic memory". Surface and diaspore bank constituents of populations of the species were investigated by means of relevés and soil. To explore temporal dynamics of genetic patterns, sampling was repeated seasonally. Genetic structure and diversity of the two stages were analysed using ISSR markers. In case of the existence of a diaspore bank capable of conserving genetic variability over generations, similar or higher levels of genetic diversity are expected in the soil than at the surface. We found that genetic diversity in the two stages was, indeed, similar. In addition, the diaspore bank contained more haplotypes, including rare ones, and entire haplotype lineages specific to the diaspore bank were detected. This suggests that the diaspore bank of *Mannia* may be able to accumulate and store genetic variability over seasons. Results showed that this may be especially important at the patch level. We postulate that the presence of a diaspore bank acting as a reservoir of genetic diversity is especially probable in bryophytes of temporarily available habitats, with long-lived spores and genetically variable populations. Further investigations comparing surface and diaspore bank parts of populations could significantly contribute to the understanding of the dynamics of bryophyte populations and to the development of more effective conservation strategies.

Keywords: diaspore bank; dynamics; genetic memory; surface; *Mannia fragrans*; seasons

Introduction

In vascular plants, seed dispersal is not always immediately followed by germination. Some seeds are capable of remaining dormant but viable in the soil for many years leading to the development of soil propagule banks (e.g. Thompson et al. 1997). Although little is known about dormancy in bryophytes to date and it is considered to be generally rare, (Miles & Longton 1992, During 1979), many species have been demonstrated to rely on a large and persistent diaspore bank. This is especially supposed to play an important role in the life of populations in habitats with great environmental fluctuations (During 1997, 2001).

As a result of the influence of the reproductive system, dispersal characteristics and habitat dynamics, genetic structure of plant populations is generally nonrandomly distributed (Loveless and Hamrick 1984). Propagule banks may have a significant impact on genetic patterns detected above ground (Levin 1990, Mc Cue and Holtsford 1998). Due to delayed germination of propagules depending on internal and/or environmental factors, seed banks may function as a kind of "genetic memory", accumulating and storing propagules formed in different years and under potentially different environmental conditions (Cabin 1996; Cabin et al. 1998). Genetic structure of the propagule bank may have a considerable effect on the genetic and demographic structure and evolutionary potential of subsequently developing populations at the surface (Cabin et al. 1998, Nunney 2002). Propagule banks may increase effective population size and may be able to restore genetic variation when the size of established populations is considerably reduced (Nunney 2002). In the same way, they may also be a source of genetic novelty, containing genotypes absent at the surface (Uehara et al. 2006). As dynamic systems, propagule banks are coupled with changes above ground and may respond to environmental variations and changes in population parameters (Koch et al. 2003). Spatial genetic structure of the propagule bank and of the populations at the surface may thus be mutually dependent (Cabin 1996, Cabin et al. 1998).

To sum up the above-mentioned issues, in the presence of a large propagule bank and/or dormant states, comparison of genetic structure at the surface and in the diaspore bank is important to better understand the dynamics and genetic history of populations. With the recent development of specific molecular markers, this has become an advancing field of seed bank research in vascular plants in the last few years (Bennington et al. 1991, Tonsor et al 1993, Cabin et al. 1998, Alvarez-Buylla et al. 1996, McCue and Holtsford 1998, Mahy et al 1999, Nunney et al. 2002, Koch et al. 2003, Barrett et al. 2005, Shimono et al. 2006). However, in order to be able to draw general conclusions about how propagule banks

influence evolutionary and genetic dynamics of populations, more empirical studies are needed.

Compared to the seed bank of vascular plants, the diaspore bank of bryophytes has received less attention so far. Nonetheless, the increased number of studies in the past ten years have resulted in extended knowledge about its composition and dynamics and general patterns found are comparable to those in higher plants (cf. During 1997, 2001 for a review). Seasonal variation in the amount of propagules stored in the diaspore bank have been demonstrated (Hock et al. 2004), but nothing is known about the influence of the diaspore bank on the genetic structure of surface populations. A parallel between seed banks and bryophyte diaspore banks in that they both may conserve considerable genetic diversity, was proposed relatively early (During 1997), but no experimental studies have yet assessed this issue. This is partly due to technical difficulties originating from the small amounts of plant material. The presence of a diaspore bank functioning as a long-term reservoir of genetic variability is especially possible in species relying on large, longer-lived diaspore banks, such as colonists or shuttle species (sensu During 1992). If a diaspore bank is able to maintain genetic diversity, similar or higher levels of genetic diversity are expected belowground compared with at the surface (Mahy et al. 1999). In addition, the accumulation of the products of several mating events should increase the amount of genetic variation stored in the soil (Barrett et al. 2005, Shimono et al. 2006).

Using *Mannia fragrans* (Balb.) Frye and Clark, a model species with a diaspore bank of considerable size (Hock 2003), we tested whether the hypothesis of "propagule bank as a genetic memory" could be applied to bryophytes as well. To do this, we analysed the genetic composition of selected populations at the surface and in the diaspore bank. Among others, we aimed to answer the following questions: (1) Are levels of genetic diversity in the diaspore bank and at the surface similar? (2) Is there any difference in samples from different seasons in terms of genetic variability?

Methods

Model species and study sites

Mannia fragrans, a thermophytic thallose liverwort is a good candidate for testing the hypothesis of genetic memory. First, it has large spores (Damsholt 2002), supposed to be capable of surviving for longer periods in the soil (Inoue 1960, Hock 2003). Along with the fact that, due to their size, most spores probably fall close to the mother plant (Söderström and Herben 1997, Crum 2001), this generally leads to the formation of a large, persistent diaspore bank (Hock 2003). Second, the species inhabits open grasslands, where the diaspore bank is supposed to play a great role in (re)colonization, due to the unpredictability of formation of suitable microsites and due to the predictable seasonal alternation of favourable versus arid periods. Finally, selected populations of the species were found to be polymorphic in earlier studies (Chapter III).

Temporal, spatial and financial constraints of the study meant that parallel investigations of only two populations could be carried out. Both populations grow in open grasslands: Population 1 on dolomite (Vértes Mountains, N 47°31'21", E 18°29'57"), and Population 2 on limestone bedrock (Mecsek Mountains, N 46°06'09", E 18°12'27", cf Chapter III and Plate 5).

Sampling and DNA analysis

In order to explore the dynamics of the genetic composition in the diaspore bank and at the surface, sampling was carried out seasonally, in periods favourable for spore germination and thallus growth. Sampling took place three times: (1) November 2004, preceding production of new spores, (2) April 2005, immediately following spore dispersal and (3) November 2005.

As the species formed distinct patches of 3–15 cm diameter in both populations investigated, all of these patches were sampled and marked. Depending on patch size, samples contained 3–5 plants/patch. Soil samples were taken from the immediate vicinity of each patch (volume ca 200 cm^3 and depth ca 3 cm). Soil samples were sieved in the laboratory to exclude living fragments of thalli. Cultivation of samples was carried out after Hock et al. (2006). Thalli germinated from spores were cultivated until they had reached a minimum size of 1 cm (usually after 3.5–4 months). After this period, all individuals were harvested and

cleaned. Cleaning of individuals from the field and from the soil samples, extraction of DNA and amplification were carried out as in Chapter III.

Since soil samples from Population 1 and Population 2 at the first and second sampling date, respectively, were subject to fungal infection and only a small number of plants emerged, these samples were excluded from further analysis.

Due to their reproducibility and reliability, ISSR markers were used to study the genetic structure of the populations (Wolfe and Liston 1998, Hassel and Gunnarsson 2003, Werner et al. 2003, Hassel et al. 2005).

Data analysis

From now on population parts at the surface and in the diaspore bank will be referred to as stages. Genetic variation at the level of sampling dates and stages was investigated with a nested analysis of molecular variance (stages nested within sampling dates; AMOVA, Excoffier et al. 1992). Levels of genetic differentiation were measured by F_{CT}, F_{SC}, and F_{ST}, referring to the differentiation among sampling dates, between stages within sampling dates and within stages, respectively. To determine levels of significance, 1000 random permutation replicates were computed.

To test for differences in allelic composition between stages at a given date and among samples from different sampling dates, an exact test of population differentiation (Raymond and Rousset 1995) was performed with the TFPGA software (TFPGA 1.3, Miller 1998), using 1000 dememorization steps, 20 batches and 2000 permutations per batch. This test applies a contingency table (Fisher's RxC test) and a Markov Chain Monte Carlo approach to determine whether significant differences in allele frequencies exist among stages or seasons. The exact test of population was also applied to test differences in haplotypic composition. For this purpose, ARLEQUIN 3.01 was used (Excoffier et al. 2005).

To compare genetic diversity of stages, standard genetic indices, i.e. number and percentage of polymorphic loci (S), average gene diversity over loci (H_s; Nei, 1987), average haplotype diversity (h_s; Nei, 1987) and occurrence of private and rare haplotypes and alleles were estimated. Analyses were performed using the ARLEQUIN 3.01 (Excoffier et al. 2005) and the GenAlEx 6 (Peakall and Smouse 2006) software packages.

To assess the significance of the differences in H_s, samples were randomised among the different stages/seasons using FSTAT 2.9.3 (Goudet 2001). H_s was then calculated from this

randomised data set. The P-value of the test reflects the proportion of randomised data sets giving a larger difference in H_s values than the observed ones.

At the patch level, exact tests of population (Raymond and Rousset 1995) differentiation were applied to test for differences in allele and haplotype frequencies between the two stages using the softwares TFPGA 1.3 (Miller 1998) and ARLEQUIN 3.01 (Excoffier et al. 2005), respectively. Test parameters for the former were the same as described for the population-level comparisons.

Results

When comparing genetic variation at different levels (Table 1), the within stage components of variance were dominant at both sites (95–98%). Only 3–8% represented variation between stages within sampling dates. There were no differences among sampling dates. Negative values obtained among them reflect that individuals from different dates were more closely related to each other than were individuals within samples from the same dates.

Average gene and haplotype diversities estimated did not differ between stages (Table 2) or sampling dates (Table 3).

	df	Variance component	Variance (%)	Fixation index	P
Population 1					
Among sampling dates	1	- 0.051	- 2.65	- 0.0266	0.305
Among stages[1] within sampling dates	2	0.156	8.06	0.07863	0.000
Within stages	137	1.826	94.59	0.05413	0.001
Total	140	1.931			
Population 2					
Among sampling dates	1	- 0.013	- 0.98	- 0.0098	0.320
Among stages[1] within sampling dates	2	0.042	3.21	0.0317	0.008
Within stages	197	1.267	97.78	0.0222	0.013
Total	200	1.296			

Table 1 Results of the nested AMOVA. [1] Stage refers to the surface and diaspore bank parts of populations.

Sampling date	N°s/n°h	S	%p	N°bands/ n°private bands	% private bands	N° bands under 5% freq.	N°private haplotypes	N° haplotypes under 5% freq.	H_S±SD	Significance (H_s)	h_S±SD
Population 1											
2/above-ground	34/13	35	79.5	43/7	16.3	0	7	6	0.120±0.066	0.369	0.139±0.092
2/diaspore bank	21/11	8	18.2	26/1	3.9	1	5	6	0.060±0.037		0.143±0.099
3/above-ground	44/13	35	71.4	44/7	15.9	18	5	7	0.069±0.040	0.904	0.136±0.089
3/diaspore bank	42/12	32	65.3	42/5	11.9	18	4	7	0.064±0.037		0.147±0.096
Population 2											
1/above-ground	45/13	37	80.4	44/18	40.9	19	6	7	0.075±0.043	0.963	0.126±0.084
1/diaspore bank	49/15	11	23.9	28/2	7.1	3	8	10	0.060±0.036		0.117±0.077
3/above-ground	59/10	9	31.0	28/3	10.7	3	3	3	0.063±0.041	0.300	0.157±0.105
3/diaspore bank	48/16	8	27.6	26/1	3.9	1	9	9	0.082±0.050		0.116±0.0764

Table 2 Comparison of genetic variation at the surface and in the diaspore bank. N°s/N°h = number of samples/number of haplotypes; S = number of polymorphic loci; %p = percentage of polymorphic loci; H_s = average gene diversity over loci; h_s = average haplotype diversity.

Sampling date	N°s/n°h	S	%p	N°bands/ n°private bands	% private bands	N° bands under 5% freq.	N°private haplotypes	N° haplotypes under 5% freq.	H_S±SD	Significance (H_s)	h_S±SD
Above-ground											
Population1											
1	43/12	50	89.3	55/9	16.4	31	4	6	0.084±0.047	0.674	0.084±0.055
2	34/13	35	62.5	43/0	0	0	3	6	0.094±0.052		0.086±0.057
3	44/13	35	62.5	44/1	2.3	18	4	7	0.061±0.035		0.084±0.055
Population 2											
1	45/13	37	69.8	44/12	27.3	19	7	8	0.065±0.037	0.733	0.068±0.045
2	44/13	24	45.3	38/6	15.8	14	7	9	0.049±0.030		0.068±0.045
3	59/10	9	17.0	28/2	7.1	3	4	3	0.035±0.022		0.065±0.044
Diaspore bank											
Population1											
2	21/11	8	18.6	26/1	3.9	1	3	8	0.062±0.038	1.000	0.122±0.081
3	42/12	32	74.4	42/17	40.5	18	4	7	0.072±0.043		0.117±0.077
Population 2											
1	49/15	11	37.9	28/3	10.7	3	5	10	0.095±0.057	0.671	0.088±0.058
3	48/15	7	24.1	26/1	3.9	1	5	9	0.081±0.050		0.088±0.058

Table 3 Genetic variability at the surface and in the diaspore bank of *Mannia fragrans*: the effect of sampling dates. N°s/N°h = number of samples/number of haplotypes; S = number of polymorphic loci; %p = percentage of polymorphic loci; H_s = average gene diversity over loci; h_s = average haplotype diversity.

Differences among stages pooled for two sampling dates					Differences among sampling dates (1,2 denote sampling dates)				
	Allelic frequency			Haplotype frequency		Allelic frequency			Haplotype frequency
	χ^2	df	p			χ^2	df	p	
Population 1	104.334	92	0.175	*	Population 1, above-ground	269.282	112	*<0.001*	1-2* only
					Population 1, diaspore bank	101.586	86	0.120	*
Population 2	490.747	84	0.999	*	Population 2, above-ground	451.098	106	*<0.001*	NS
					Population 2, diaspore bank	140.564	58	*<0.001*	*

Table 4 Differences in allele and haplotype frequencies between stages and sampling dates. * = p<0.05, NS = non-significant.

No significant difference was found between stages on the basis of allele frequencies either (Table 4).

However, allele frequencies showed significant differentiation between sampling dates in both stages, with the exception of soil samples from Population 1. Haplotype frequencies differed significantly between stages (Table 4).

In spite of the smaller samples size of the diaspore bank, compared to that of the surface, the former had slightly more haplotypes (total percentage of different haplotypes at the surface and in the diaspore bank: 33.3–36.5; 22.1–32.0 for Population 1 and 2, respectively), including more private (15.38–14.29; 8.65–17.53) and rare haplotypes (16.67–

20.64; 9.62–19.59). A few haplotype lineages (lineage designating haplotypes occurring with frequencies above 5%) were specific to the diaspore bank (Table 5), whereas no haplotype lineages specific to the surface were found. Otherwise, the distribution patterns of haplotypes in the two stages were similar with a few dominant and numerous less frequent ones (Table 5). Between sampling dates, samples from the surface generally did not differ in haplotype frequencies (Table 4). In contrast, samples from the diaspore bank showed significant differences between sampling dates.

Population 1			Population 2		
Haplotype name	Above ground	Diaspore bank	Haplotype name	Above ground	Diaspore bank
A	23,1	11,11	a	18,27	17,53
B	14,1	17,46	b	9,62	12,37
C	11,54	9,52	c	9,62	5,16
D	11,54	4,76	d	6,7	4,12
E	11,54	3,18	e	3,85	4,12
F	5,13	–	f	2,88	9,28
G	5,13	7,94	g	0,96	2,06
H	3,85	11,11	h	0,96	–
I	2,56	–	i	0,96	–
J	2,56	7,94	j	0,96	–
K	1,28	–	k	0,96	–
L	1,28	–	l	0,96	–
M	1,28	–	m	0,96	–
N	1,28	–	n	0,96	–
O	1,28	–	o	–	***8,25***
P	1,28	–	p	–	2,06
Q	1,28	–	q	–	1,03
R	–	***7,94***	r	–	2,06
S	–	1,59	s	–	1,03
T	–	1,59	t	–	1,03
U	–	4,76	u	–	2,06
V	–	***6,35***	v	–	1,03
W	–	1,59	w	–	1,03
X	–	3,18	x	–	1,03

Table 5 Haplotypic composition of surface and diaspore bank populations of *Mannia fragrans*. Data given in percentages and pooled for two sampling dates (2-3 in case of Population 1 and 1-2 in Population 2). Italics: stage specific haplotype lineages (lineage=haplotypes occurring with frequencies above 5%).

	Population differentiation			
	Allele frequencies			Haplotype frequencies
Patch	χ^2	df	P	P<0.05
1	*7.9755*	*10*	*0.6312*	
2	*25.3299*	*12*	*0.0133**	*
3	*4.6172*	*12*	*0.9696*	
4	*25.9229*	*10*	*0.0038**	*
5	*16.1404*	*22*	*0.8089*	
6	*13.3089*	*14*	*0.5024*	
7	*11.9264*	*12*	*0.4516*	
8	*4.5592*	*12*	*0.9711*	
9	*78.7051*	*86*	*0.6991*	
10	*3.1246*	*8*	*0.9263*	
11	*12.1029*	*10*	*0.2782*	*

* = P<0.05

Table 6 Differences in allele and haplotype frequencies between stages at the patch level.

At the patch level, allele and haplotype frequencies were either similar in the two stages, or, in a few patches, significant differences were detected (Table 6). In the case of haplotypes, these differences were mainly due to the additional presence of rare haplotypes in the diaspore bank.

Discussion

As a complement to the dispersal of seeds and genes over space, propagule banks are capable of dispersing them through time (Venable and Brown 1988, Baskin and Baskin 1998) as a bet-hedging adaptation to environmental uncertainty. This may result in differences in the genetic composition of propagule banks and surface populations (Templeton and Levin 1979). Accordingly, allele frequencies of the propagule bank and adult populations may differ considerably (Tonsor et al 1993, Cabin 1996). Patterns detected by existing studies range from higher polymorphism in the propagule bank (McCue and Holtsford 1998, Morris et al. 2002) to higher genetic diversity above ground (Cabin et al. 1998). In our case, allele and haplotype diversities were similar in the diaspore bank and in surface populations. Analogous results were found in some studies on vascular plants (Mahy et al. 1999, Koch et al. 2003). Similarity of the two stages, together with the relatively constant patterns over seasons indicates, that the diaspore bank may be able to conserve genetic diversity between generations (Mahy et al. 1999). In case of a shorter-lived diaspore bank (spores viable for less than one year) one would expect to find less genetic diversity in the soil than above ground. The fact that the diaspore bank and the surface did not substantially differ from each other may also reflect the effect of regular yearly spore rain from surface populations (Koch et al. 2003).

Allelic richness of populations is relatively low in *Mannia fragrans*, and the formation of new alleles is supposed to be rare (Chapter III). Although allele frequencies differed between sampling dates at the surface, this probably represents the result of randomly occurring, rare somatic mutations (Chapter III), rather than reflecting general trends. This interpretation is supported by the distribution of rare and stage-specific alleles occurring concentratedly in a few individuals only, which leads to the lack of significant differences in haplotype frequencies among sampling dates. These results suggest that overall genetic structure at the surface is more or less constant over seasons. In the diaspore bank, additional significant differences in haplotype frequencies between sampling dates may reflect the effect of additional spore input between them. Recombination may create haplotypes containing new combinations of existing alleles. These new haplotypes have little chance to germinate readily at the surface (no significant difference in haplotype frequencies among sampling dates were observed), due to elimination of rare new haplotypes by genetic drift (Chapter III). However, the present results suggest that they may be conserved in the soil.

The closely related general distribution patterns of haplotypes in the two stages is indicative of a mutual relationship between them. Although here again, no differences in allelic frequencies were detected between the two stages, probably due to the low allelic richness in populations (Chapter III), the way existing alleles were combined (haplotypes) provided valuable information. As haplotypic richness tended to be higher in the soil and haplotype lineages specific to the diaspore bank were detected, we suppose that the diaspore bank of the species does not only conserve genetic variability from one generation to another: it may even be able to accumulate the results of several mating periods (Barrett et al. 2005). Haplotype lineages specific to the diaspore bank could have been present at the surface earlier and may have disappeared later on. If so, the diaspore bank of *Mannia* may be able to conserve and restore genetic variability lost from the surface, as predicted formerly in flowering plants (del Castillo 1994, etc.). Haplotype lineages specific to the diaspore bank may also have originated from remote sites, however this is less probable due to the low long-range dispersal ability of the spores, as proposed in Chapter III. Little is known about the longevity of spores of *Mannia fragrans*. Experimental studies (Inoue 1960) found no germination of spores under "ordinary herbarium conditions" after 18 months. However, the number of replicates is not specified in this work, and it was probably rather low, since only two herbarium accessions were used. How longevity under such conditions compares to longevity in the field is also unclear. Our own observations in soil samples stored for different periods suggest that spores may be viable for periods longer than a year (Hock 2003). The presence of more haplotypes in the diaspore bank, including more rare and private haplotypes, as well as diaspore bank-specific haplotype lineages suggests that new, rare haplotypes formed by recombination are not definitively eliminated from the populations. Although immediate germination of such spores may be inhibited by a range of processes including spores falling into their own patch, crowding and potential inhibition through chemical components (Chapter III), they may remain viable for longer periods in the diaspore bank. As further confirmed by results from the analyses at the patch level, this may play an important role in the fine-scale dynamics of the species.

Spatial structure of propagule banks and reproducing plants may be related to eachother, as suggested by the results of comparative studies analysing the density of propagule rain in relation to distance from the mother plant (Wyatt 1977, Miles and Longton 1992, Stoneburner et al. 1992, Crum 2001). However, even in vascular plants, the spatial genetic structure of propagule banks has received little attention so far (Cabin 1996, Cabin 1998), with only a few studies investigating spatial associations between propagule bank and reproducing plants

(Shimono et al. 2006, Schneller 1999). In our study, analysis of fine-scale patterns revealed interesting differences at the patch level. Soil samples showed similar amounts of different alleles and haplotypes as patches above ground, and, in some cases, even more. This seems to confirm the importance of a diaspore bank in local, fine-scale dynamics and conservation of genetic variability, probably related to the supposed relatively short spore dispersal distances. As *Mannia fragrans* is a shuttle strategist (Vojtkó 1998, During 1992), specialized on periodically but spatially unpredictably appearing, short-lived microsites (During 1992), its diaspore bank may play a great role in colonization of newly formed microsites within established populations, allowing genotypes different from those in neighbouring patches to appear. Since most of the large spores of the species are supposed to fall within the own patch (Miles and Longton 1992, Söderström and Herben 1997), where chances for germination are lowered by the numerous, intermingled thalli, the diaspore bank may be essential in escaping from crowding and sib competition (Venable and Brown 1988, Baskin and Baskin 1998). These findings support the hypothesis suggested by earlier models about the important role of propagule banks particularly for shorter-lived species occurring in unpredictably appearing, rare microhabitats (Baskin and Baskin 1998).

Similar levels of genetic diversity in the diaspore bank to those found above ground, and slightly more haplotypes stored in the soil suggest that the diaspore bank of *Mannia fragrans* may play an important role in conserving local genetic diversity. As demonstrated in other species (e.g. del Castillo 1994), it probably has the potential to buffer effects of bottlenecks and to restore genetic variation lost at the surface. Since the species is specialized on temporarily available open patches, this may be especially important at the level of the unpredictably appearing microsites. As our study is the first one to investigate genetic structure of the bryophyte diaspore bank, it is hard to infer general trends applying to all bryophytes. Smaller spore size is supposed to be coupled with shorter life-span (Crum 2001) and the majority of bryophytes have small (5 to about 200 μm, but generally under 30 μm, Smith 2004, Glime 2006) spores containing little storage material (Miles and Longton 1990, During 1997). This indicates that a diaspore bank may function as a long-term pool of genetic variability relatively rarely and its role as a genetic reservoir is more likely in species with larger spores. However, although experimental evidence for spore longevity in the field is sparse (Miles and Longton 1990, During 1986, Sundberg and Rydin 2000), the capacity of bryophyte spores to establish from a persistent spore bank has been demonstrated (Furness and Hall 1981, Clymo and Duckett 1986, Jonsson 1993, During 1997). Its relation to spore longevity in the field may not be clear, but evidence of spore longevity from material stored

in herbaria also exists (Crum 2001) and even species with smaller spores (under 30 μm) remain viable for longer, sometimes for considerably long periods (e.g. Hoffman 1970, Chalaud 1932). These data suggest that for periods of time, in the range of a few years, the diaspore bank may have the potential to conserve genetic variability.

Spore size is not the only possible correlative of spore longevity in the soil. In addition, spores of considerable size may have extremely short life-span, as in *Conocephalum conicum* (Inoue 1960) and vice-versa (*Dicranella*: Chalaud 1932). Thus, when considering an ideal candidate for the investigation of the role of the bryophyte diaspore bank as a genetic memory, habitat and life strategy should also be taken into account. Species with shorter life cycles (colonists and shuttle species, sensu During 1992) occurring in periodically available (micro-)habitats are shown to usually rely on a larger diaspore bank (During 1997, 2001). We thus postulate that storage of genetic diversity in the diaspore bank is most likely to occur in such species. Patterns of distribution of genetic variability in the diaspore bank and in surface populations of *Mannia fragrans* are probably not unique. As life history is known to considerably influence genetic structure of populations (Loveless and Hamrick 1984, Nybom 2004), similar trends may be expected in species with similar life history traits.

Finally, the ability of the bryophyte diaspore bank to conserve important genetic variability also depends on genetic variability of established populations (thus on the frequency of sexual reproduction producing new genetic combinations or on the frequency of mutations). In species where surface populations show low genetic variability, it may be difficult to decide whether the potential similarity of the diaspore bank to the surface populations reflects transient nature of the former or whether it is only a consequence of the low genetic variability. Liverworts, in general, are expected to be genetically less variable than mosses (Wyatt 1994), thus the latter may be better candidates for future testing of the hypothesis of "genetic memory".

Spore dispersal distances may determine the scale at which the diaspore bank plays a role as a reservoir of genetic diversity. Since most large bryophyte spores are supposed to be long-lived (Crum 2001) and spore dispersal distances decrease with increasing spore size (van Zanten 1978, van Zanten and Gradstein 1988, Miles and Longton 1992, Söderström and Herben 1997), it is probable that the role of the bryophyte diaspore bank in conserving genetic diversity is more important in local dynamics, at a fine scale.

Acknowledgements

This research has been supported by the Hungarian Scientific Research Fund (OTKA-T047156). Aelys Humphreys is gratefully acknowledged for revising the language of the manuscript.

References

Alvarez-Buylla ER, Chaos Á, Piñero D, Garay AA 1996 Demographic genetics of a pioneer tropical tree species: patch dynamics, seed dispersal, and seed banks. *Evolution* **50**: 1155-1166

Barrett LG, He T, Lamont BB, Krauss SL 2005 Temporal patterns of genetic variation across a 9-year-old aerial seed bank of the shrub *Banksia hookeriana* (Proteaceae). *Mol Ecol* **14**: 4169-4179

Baskin CC, Baskin JM 1998 Seeds. *Ecology, biogeography, and evolution of dormancy and germination.* Academic Press, San Diego, CA, USA

Bennington CC, McGraw JB, Vavrek MC 1991 Ecological genetic variation in seed banks. II. Phenotypic and genetic differences between young and old subpopulations of *Luzula parviflora*. *J Ecol* **79**: 627-643

Cabin RJ 1996 Genetic comparison of seed bank and seedling population of the perennial desert mustard, *Lesquerella fendleri*. *Evolution* **50**: 1830-1841

Cabin RJ, Mitchell RJ, Marshall DL 1998 Do surface plant and soil seed bank populations differ genetically? A multipopulation study of the desert mustard *Lesquerella fendleri* (Brassicaceae). *Am J Bot* **85**: 1098-1109

Chalaud G 1932 Germination des spores et phase protonématique. In: Verdoorn F (ed) *Manual of bryology.* Martinus Nijhoff, The Hague

Clymo RS, Duckett JG 1986 Regeneration of *Sphagnum*. *New Phytol* **102**: 589-614

Crum H 2001 *Structural diversity of bryophytes.* University of Michigan Herbarium, Ann Arbor

Damsholt K 2002 *Illustrated flora of Nordic liverworts and hornworts.* Nordic Bryological Society, Lund, Sweden

del Castillo RF 1994 Factors influencing the genetic structure of *Phacelia dubia*, a species with a seed bank and large fluctuation in population size. *Heredity* **72**: 446-458

During HJ 1979 Life strategies of bryophytes: a preliminary review. *Lindbergia* **5**: 2-18

During HJ 1986 Longevity of spores of *Funaria hygrometrica* in chalk grassland soil. *Lindbergia* **12**: 132-134

During HJ 1992 Ecological classifications of bryophytes and lichens In: Bates JW, Farmer AM (eds) *Bryophytes and lichens in a changing environment.* pp 1-31. Oxford University Press, Oxford

During HJ 1997 Bryophyte diaspore banks. *Adv Bryol* **6**: 103-134

During HJ 2001 Diaspore banks. *Bryologist* **104**: 92-97

Excoffier L, Laval G, Schneider S 2005 Arlequin (version 3.0): An integrated software package for population genetics data analysis. Evol Bioinf Online 1: 47-50

Excoffier L, Smouse P, Quattro J 1992 Analysis of molecular variance inferred from metric distances among DNA haplotypes: Application to human mitochondrial DNA restriction data. *Genetics* **131**:479-491

Furness SB, Hall RH 1981 An explanation of the intermittent occurrence of *Physcomitrium sphaericum* (Hedw.) Brid. *J Bryol* **11**: 733-744

Glime, J. M. 2006 *Bryophyte Ecology. Vol 1. Physiological Ecology.* http://www.bryoecol.mtu.edu/ [Accessed 13 February 2007].

Goudet J (2001) *FSTAT, a program to estimate and test gene diversities and fixation indices (*Version 2.9.1*).* Available from http://www2.unil.ch/popgen/softwares/fstat.htm

Hassel K, Gunnarson U 2003 The use of inter simple sequence repeats (ISSR) in bryophyte population studies. *Lindbergia* **28**: 152-157

Hassel K, Såstad SM, Gunnarsson U, Söderström L 2005 Genetic variation and structure in the expanding moss *Pogonatum dentatum* (Polytrichaceae) in its area of origin and in a recently colonized area. *Am J Bot* **92**: 1684-1690

Hock Zs 2003 *A kriptogám diasprórabank vizsgálata dolomit sziklagyepekben.* [The diaspore bank of cryptogams in grasslands on dolomite rock.] MSc thesis, Eötvös Loránd University, Department of Plant Taxonomy and Ecology, Budapest, Hungary

Hock Zs, Szövényi P, Tóth Z 2004 Seasonal variation in the bryophyte diaspore bank of open grasslands on dolomite rock. *J Bryol* **26**: 285-292

Hock Zs, Szövényi P, Tóth Z 2006 Seasonal variation in the spore bank of ferns in grasslands on dolomite rock. Plant Ecol 187: 289-296

Hoffman GR 1970 Spore viability in *Funaria hygrometrica*. *Bryologist* **73**: 634-635

Inoue H 1960 Studies in spore germination and the earlier stages of gametophyte development in the Marchantiales. *J Hattori Bot Lab* **23**: 148-191

Jonsson BG 1993 The bryophyte diaspore bank and its role after small-scale disturbance in a boreal forest. *J Veg Sci* **4**: 819-826

Koch M, Huthmann M, K-G Bernhardt 2003 *Cardamine amara* L. (Brassicaceae) in dynamic habitats: Genetic composition and diversity of seed bank and established populations. *Basic Appl Ecol* **4**: 339-348

Levin DA 1990 The seed bank as a source of genetic novelty in plants. *Am Nat* **135**: 563-572

Loveless MD, Hamrick JL 1984 Ecological determinants of genetic structure in plant populations. *Ann Rev Ecol Syst* **15**: 65-95

Mahy G, Vekemans X, Jacquemart A 1999 Patterns of allozymic variation within *Calluna vulgaris* populations at seed bank and adult stages. *Heredity* **82**: 432-440

McCue KA, Holtsford TP 1998 Seed bank influences genetic on genetic diversity in the rare annual *Clarkia springvillensis* (Onagraceae). *Am J Bot* **85**: 30-36

Miles CJ, Longton RE 1990 The role of spores in reproduction in mosses. *Bot J Linn Soc* **104**: 149-173

Miles CJ, Longton RE 1992 Deposition of moss spores in relation to distance from parent gametophytes. *J Bryol* **17**: 355-368

Miller M.P 1998 *TFPGA (Tools For Population Genetic Analysis) Version 1.3*. Department of Biological Sciences, Northern Arizona University, Arizona

Morris AB, Baucom RS, Cruzan MB 2002 Stratified analysis of the soil seed bank in the cedar glade endemic *Astragalus bibullatus*: evidence for historical changes in genetic structure. *Am J Bot* **89**: 29-36

Nunney L 2002 Effective size of annual plant populations: the interaction of a seed bank with fluctuating population size in maintaining genetic variation. *Am Nat* **160**: 195-204

Nybom H 2004 Comparison of different nuclear DNA markers for estimating intraspecific genetic diversity in plants. *Mol Ecol* **13**: 1143-1155

Peakall R, Smouse PE 2006 GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. *Mol Ecol Notes* **6**: 288-295

Raymond M, Rousset F 1995 An exact test for population differentiation. *Evolution* **49**: 1280-1283

Schneller JJ 1999 How much genetic variation in fern populations is stored in the spore banks? A study of *Athyrium filix-femina* (L) Roth. *Bot J Linn Soc* **127**: 195-206

Shimono A, Ueno S, Tsumura Y, Washitani I 2006 Spatial genetic structure links between soil seed banks and above-ground populations of *Primula modesta* in subalpine grassland. *J Ecol* **94**: 77-86

Smith AJE 2004 *The moss flora of Great Britain and Ireland.* Cambridge University Press, Cambridge

Söderström L, Herben T 1997 Dynamics of bryophyte metapopulations. *Adv Bryol* **6**: 205-240

Stoneburner A, Lane DM, Anderson LE 1992 Spore dispersal distances in *Atrichum angustatum* (Polytrichaceae). *Bryologist* **95**: 324-328

Sundberg S, Rydin H 2000 Experimental evidence for a persistent spore bank in *Sphagnum*. *New Phytol* **148**: 105-116.

Templeton AR, Levin DA 1979 Evolutionary consequences of seed pools. *Am Nat* **114**: 232-249

Tonsor SJ, Kalisz S, Fisher J, Holtsford TP 1993 A life-history based study of population genetic structure: seed banks to adults in *Plantago lanceolata*. *Evolution* **47**: 833-843

Thompson K, Bakker JP, Bekker RM 1997 *The soil seed banks of North West Europe: methodology, density and longevity.* Cambridge University Press, Cambridge

Uehara K, Tanaka N, Momohara A, Zhou Z-K 2006 Genetic diversity of an endangered aquatic plant, *Potamogeton lucens* subspecies *sinicus*. *Aqu Bot* **85**: 350-354

Van Zanten BO 1978 Experimental studies on trans-oceanic long-range dispersal of moss spores in the southern hemisphere. *J Hattori Bot Lab* **44**: 455–482

Van Zanten BO, Gradstein SR 1988 Experimental dispersal geography of neotropical liverworts. *Beiheft Nova Hedw* **90**: 41–94

Venable DL, Brown JS 1988 The selective interactions of dispersal, dormancy, and seed size as adaptations for reducing risk in variable environments. *Am Nat* **131**: 360-384

Vojtkó A 1998 Relationships between the morphology of the spores of Hungarian Hepaticae species and their strategy types. *Studia Bot Hung* **27-28**: 109-115

Wolfe AD, Liston A 1998 Contributions of PCR-based methods to plant systematics and evolutionary biology. In: Soltis DE, Soltis PS, Doyle JJ (eds) *Plant Molecular Systematics II*. pp 43-86. Kluwer

Wyatt R 1977 Spatial pattern and gamete dispersal distances in *Atrichum angustatum*, a dioicous moss. *Bryologist* **80**: 284-291

Wyatt R 1994 Population genetics of bryophytes in relation to their reproductive biology. *J Hattori Bot Lab* **76**: 147-157

General summary

The present thesis assesses questions related to the ecology and population biology of bryophytes and ferns living in grasslands on dolomite rock, focusing on two main issues. First, the composition and dynamics of the diaspore bank of bryophyte and fern populations is studied, with special emphasis on the life history traits of its components and changes caused by seasonal variations in environmental conditions (Chapter I-II). Second, the population genetic consequences of the reproductive ecology of a model species, *Mannia fragrans*, are explored using ISSR markers (Chapter III-IV).

The first chapter investigates the bryophyte diaspore bank in adjacent grasslands on dolomite rock with fundamentally different structure and microclimate. I tested the effect of habitat type on the composition of the bryophyte vegetation and diaspore bank with the aid of relevés and incubation of soil samples taken using a permanent grid. Seasonal dynamics of species in the diaspore bank and correlations with changes in the above-ground bryophyte vegetation was studied by sampling during different seasons. Special attention was paid to life-history strategies of individual species in the interpretation of patterns obtained. Bryophyte cover, species composition and dominating life-history strategies, both above ground and in the diaspore bank, were significantly associated with habitat type. Seasonal changes in the total cover of bryophytes above-ground were reflected in the diaspore bank as well: patterns detected were generally coupled, confirming the role of the diaspore bank as an important resource for bridging unfavourable periods. Reaction of species to seasonal changes differed according to life-strategy. However, sometimes species assigned to the same strategy type behaved differently, which proves the heterogeneity of established categories.

In the second chapter I studied composition and seasonal dynamics of the spore bank of ferns from the same sites taking environmental changes and spore dispersal periods into account. Additionally, life span of fern spores stored in the soil was estimated through storage of soil samples. The grasslands investigated housed only some scattered individuals of *Asplenium ruta-muraria*. However, cultivation of prothallia emerging from soil samples revealed that in lower amounts spores of several species, partly from remote sites, are present in the soil. Similarly to bryophytes, seasonal variations in environmental conditions caused significant changes in the composition of the spore bank of the dominating *Asplenium ruta-muraria*. The amount of prothallia in soil samples was highest at the time of spore dispersal. According to storage experiments, spores of the species are able to remain viable for at least one year, and some of them may be dormant.

The third chapter concentrates on the model species *Mannia fragrans* frequently occurring in open grasslands. It is a thallose liverwort with a polyoicous reproductive system (i.e. populations containing female, male and bisexual plants), yearly sexual reproduction and frequent clonal propagation by fragmentation. One aim of the study was to understand the functioning of its poorly understood reproductive system. Factors influencing sex expression, the proportion of uni- and bisexual individuals and the two sexes, and clonal traits related to sex state were assessed. The second part of the study focused on the population genetic fingerprints of the reproductive system using ISSR markers, in order to better understand the relative importance of its elements. Sex expression was higher in larger, probably older patches, containing numerous, small intermingled thalli with short ventral branches. Patches with a few individuals only, probably new colonizations, mostly contained larger plants that did not express sex. These plants had more bifurcations and less ventral branches than males and females. Bisexual thalli were rare, their clonal traits and microhabitat being similar to non-expressing plants. This suggests that colonizers first developing organs of both sexes are selected to ensure sexual reproduction when no reproductive partner is present. Sex ratios were female biased and males showed more input into clonal growth after reproduction than females. However, this could not clearly be assigned to higher costs of reproduction in the latter. In spite of the frequent spore production and the negligible role of intra-gametophytic selfing, crossing turned out to often occur between genetically identical thalli, producing spores functionally equivalent to asexual propagules. Together with the frequent clonal propagation by fragmentation, this contributed to the relatively low amount of genetic diversity detected. Rare alleles probably formed by somatic mutation and rare recombinant haplotypes originating from recombination have little chance to spread since large spores probably mainly fall into the own patch. In addition, due to isolated state and small size of populations, they are probably prone to strong genetic drift. Genetic differences between remote populations suggest low gene flow among them.

Using the same model species, the fourth chapter investigates, whether, similarly to vascular plants, the diaspore bank of bryophytes may serve as a long term reservoir of genetic variability. In case of a diaspore bank functioning as a kind of “genetic memory”, similar or greater amounts of genetic diversity are expected in diaspore bank populations compared to the surface. In *Mannia*, no differences in allele and haplotype diversity were found among the two stages but haplotype frequencies differed clearly among them. In contrast to populations at the surface, haplotype lineages specific to the diaspore bank were detected, and diaspore bank populations also had slightly more private and rare haplotypes. The results indicate that

diaspore bank of bryophytes is very probably able to conserve genetic variation over generations. The presence of haplotypic lineages specific to the diaspore bank suggests that the diaspore bank may be important in restoring lost genetic variation. Patterns detected at the patch level show that the role of the diaspore bank as a reservoir of genetic variability may especially be important in fine-scale dynamics of this species specialized on randomly appearing, relatively short-lived microsites. Whether the diaspore bank might play a similar role in other bryophyte species depends on several factors including spore size and longevity, life history strategy, habitat characteristics and genetic variability of populations.

Results of the present thesis emphasize the need of more extended knowledge supported by empirical evidence on the elements of the life history of individual species. Particularly in endangered species or habitats, the diaspore bank may play a great role in preventing extinction or dramatic loss of genetic variability. Experimental and field studies on the reproductive ecology of species may reveal hidden elements that could be crucial for a better understanding of the life cycle. In practice, investigation of the above-mentioned issues may contribute to the development of more effective, species-specific conservation strategies. The aims of future research are: (1) to extend investigations on the influence of the diaspore bank on the genetic structure of surface populations to other bryophyte groups in order to be able to draw conclusions about its general importance (2) to conduct experimental and field studies to test still unexplored elements of the life-cycle of *Mannia fragrans* such as maximum life-span of individual thalli, spore dispersal distances and potential dormancy of spores (3) to investigate the time frame and importance of dynamics of patches within populations.

Zusammenfassung

In der vorliegenden Dissertation werden Fragen zur Populationsökologie und Reproduktionsbiologie der Moose und Farne behandelt. Dabei gibt es zwei Schwerpunkte: zuerst wird die Zusammensetzung und Dynamik der Diasporenbank beider Pflanzengruppen studiert, die Lebensgeschichte der Arten berücksichtigt und den Einfluss jahreszeitlicher Veränderungen der Umweltbedingungen erforscht (Kapitel I und II). Zweitens werden die populationsgenetischen „Fingerabdrücke“ des Fortpflanzungs-Systems der Modellart *Mannia fragrans* mittels ISSR-Markern erforscht (Kapitel III und IV).

Im ersten Kapitel wird die Moosdiasporenbank von zwei angrenzenden Dolomitfelsrasen mit sehr unterschiedlicher Struktur und verschiedenem Mikroklima untersucht. Mittels Aufnahmen im Gelände und Inkubation von Bodenproben wollte ich testen, ob die unterschiedlichen Eigenschaften der Habitate Einfluss haben auf die Artenzusammensetzung auf der Oberfläche und in der Diasporenbank. Um die jahreszeitliche Dynamik der Diasporenbank und ihre Abhängigkeit von Veränderungen der Vegetation auf der Oberfläche zu verfolgen, wurde die Probenahme aus Dauerflächen mehrmals wiederholt. Um allgemeine Trends feststellen zu können, wurden die Lebensstrategien einzelner Arten bei der Auswertung der Daten besonders berücksichtigt. Die Gesamtdeckung der Moose, die Artenzusammensetzung und die dominierenden Strategien zeigen eine deutliche Korrelation mit dem Habitattyp. Jahreszeitliche Veränderungen der Gesamtdeckung der Moose auf der Oberfläche waren mit jenen in der Diasporenbank verknüpft, was die Rolle der Diasporenbank als temporäre Zuflucht in ungünstigen Perioden bestätigt. Arten mit ähnlichen Lebensstrategien haben meist ähnlich auf die jahreszeitlichen Veränderungen reagiert. Arten, die zur selben Lebensstrategie gerechnet werden, zeigten aber nicht immer das gleiche Verhalten. Dies bestätigt die Heterogenität der heute gebräuchlichen Kategorien.

Im zweiten Kapitel studiere ich die Zusammensetzung und Dynamik der Farnsporenbank in den selben Untersuchungsgebieten. Bei der Probenahme habe ich die jahreszeitlichen Veränderungen der Umweltbedingungen und die Periode der Sporenausbreitung einzelner Arten berücksichtigt. Um Lebensdauer der Sporen zu testen, sind die entnommenen Bodenproben gelagert worden. Obwohl die untersuchten Habitate nur eine einzige Art, *Asplenium ruta-muraria*, beherbergten, sind in den Bodenproben Prothallien mehrerer Arten, teils aus weiter entfernten Habitaten in kleiner Menge erschienen. Ähnlich wie bei den Moosen, ist die Sporenbank des dominierenden *Asplenium ruta-muraria* von den Jahreszeiten abhängig. Am meisten Prothallien erschienen unmittelbar nach der Haupt-

Sporulationszeit im Sommer. Nach den Lagerungsexperimenten können die Sporen der Art ihre Keimfähigkeit mindestens ein Jahr lang behalten und ein Teil dieser Sporen ist vermutlich auch dormant.

Das dritte Kapitel befasst sich mit einer in ungarischen Trockenrasen relativ häufigen Modellart *Mannia fragrans*. Es handelt sich um ein thallöses, polyözisches Lebermoos mit jährlicher Sporenbildung und kräftiger Vermehrung durch Teilung der Thalli. Das erste Ziel meines Studie war, das wenig bekannte Fortpflanzungs-System der Art besser zu verstehen. Dazu wurden die Faktoren, welche die Bildung von Gametangien beeinflussen, der Anteil zwitteriger Individuen und das Verhältnis der Geschlechter in den Populationen untersucht. Zudem wurden individuelle Pflanzen auf geschlechtspezifische Merkmale ihres klonalen Wachstums geprüft. Ein weiteres Ziel war mittels ISSR-Markern die Konsequenzen des Reproduktions-Systems auf die genetische Struktur der Populationen zu verfolgen, um die relative Bedeutung seiner Komponenten zu bestimmen. Zahlreiche kleine Pflanzen mit kurzen seitlichen Ästen in grösseren, vermutlich älteren Flecken, tragen häufiger Gametangien. In kleineren, vermutlich jüngeren Thallus-Flecken mit nur wenigen Individuen sind die Pflanzen meistens steril und haben zahlreiche Verzweigungen aber nur wenige seitliche Äste. Der Anteil zwitteriger Pflanzen ist gering, die Merkmale ihrer Klone und ihr Mikrohabitat ähnlich wie bei sterilen Individuen. Dies deutet darauf hin, dass die Selektion Besiedler neu entstandener Mikrohabitate begünstigt, die anfänglich Organe beider Geschlechter entwickeln und damit die sexuelle Fortpflanzung auch in der Abwesenheit eventueller Partner ermöglichen. Das Geschlechtsverhältnis ist zugunsten weiblicher Pflanzen verschoben. Nach der Fortpflanzung investieren männliche Pflanzen mehr in klonales Wachstum als weibliche investiert. Es konnte aber nicht mit Sicherheit festgestellt werden, ob dies auf die höheren Kosten der Fortpflanzung bei weiblichen Pflanzen zurückzuführen ist. Trotz der häufigen Sporenbildung und der vernachlässigbaren Rolle von Selbstbefruchtung, erfolgt die Kreuzung in den meisten Fällen zwischen Individuen, die genetisch identisch sind, was zur Bildung von Sporen führt, die funktional asexuellen Propagulen gleichwertig sind. Dies hat, zusammen mit der häufigen Vermehrung durch Teilung der Klone, zur beobachteten geringen genetischen Diversität beigetragen. Seltener entstehen auch neue Allele und Genotypen durch somatische Mutation und Rekombination, aber diese haben wahrscheinlich nur geringe Chancen, sich in der Population auszubreiten, weil die Mehrheit der grossen Sporen sehr nah bei der Mutterpflanzen liegen bleiben. Zudem kann die seltene neue Variabilität durch Gendrift eliminiert werden, da die Populationen klein und isoliert sind. Unterschiede in der genetischen Struktur der untersuchten Populationen deuten auf geringen Genfluss hin.

Im vierten Kapitel habe ich ebenfalls *Mannia fragrans* als Modellart benützt um zu testen ob, ähnlich wie bei Gefässpflanzen, die Diasporenbank von Moosen ebenfalls als ein genetisches Reservoir angesehen werden kann. Wenn die Diasporenbank von *Mannia fragrans* eine Rolle als „genetisches Gedächtnis“ spielt, sollte ihre genetische Diversität gleich oder höher sein als auf der Oberfläche. Die Haplotyp- und Allel-Diversität innerhalb Populationen auf der Oberfläche und in der Diasporenbank zeigten keine Unterschiede. Hingegen waren Haplotyp- und Allelfrequenzen unterschiedlich, mit mehr seltenen und einigen häufigeren Haplotypen spezifisch für die Diasporenbank. Die Ergebnisse der Studie zeigen, dass die Moosdiasporenbank sehr wahrscheinlich fähig ist genetische Vielfalt über Generationen zu konservieren. Das Vorkommen häufigeren Haplotypen spezifisch für die Diasporenbank deutet darauf hin, dass sie eine wichtige Rolle bei der Wiederherstellung verlorener genetischen Variabilität spielen kann. Für Arten, die, wie *Mannia*, auf relativ kurzlebige Mikrohabitate, die zufällig auftreten, spezialisiert sind, ist diese Rolle wohl noch wichtiger. Ob die Diasporenbank anderer Moosarten auch eine ähnliche Rolle spielen könnte, hängt von verschiedenen Faktoren ab, wie zum Beispiel der Grösse und Lebensdauer der Sporen, der Lebensstrategie, den Eigenschaften der Habitat und der genetischen Variabilität der Populationen.

Die Ergebnisse meiner Dissertation unterstreichen das Bedürfnis einer experimentell unterstützten, vertiefteren Kenntnis über die Komponenten des Lebenszyklus einzelner Arten. Vor allem bei bedrohten Arten und Habitaten dürfte die Diasporenbank eine grosse Bedeutung haben, indem sie den Verlust oder die dramatische Reduktion von genetischen Variabilität verhindert. Gleichzeitige Labor- und Feldversuche können neue, bisher unbekannte Elemente der reproduktiven Ökologie erkennen lassen. Sie sind deswegen für ein besseres Verständnis des Lebenszyklus äusserst wichtig. In der Praxis könnten solche Untersuchungen wesentlich zur Entwicklung wirksamerer Strategien zur Erhaltung von seltenen Arten beitragen. Zukünftige Untersuchungen werden sich auf den folgenden Fragen konzentrieren. (1) Wie gross ist die allgemeine Bedeutung der Diasporenbank für die Populationsdynamik und für die Erhaltung genetischer Vielfalt bei Moosen? (2) Wie lange können einzelne Thalli und Sporen von *Mannia fragrans* überleben? Sind die Sporen dormant? (3) Wie lange sind einzelne *Mannia* Flecken vorhanden und wie ist ihre zeitliche Dynamik in den Populationen?

Lebenslauf

Personalien

Name und Vorname:	Hock, Zsófia
Geboren am:	24. Mai 1979 in Budapest, Ungarn
Heimatort:	Budapest, Ungarn

Ausbildung

Mittelschule:	Városmajori Gimnázium, Budapest Abschluss 1998, Matura
Hochschulstudium:	Eötvös Loránd Universität, Wissenschaftliche Fakultät, Fachübersetzerin in Französisch, 1998 bis 2003
	Eötvös Loránd Universität, Wissenschaftliche Fakultät, Fachbiologin, 1998 bis 2003
	Eötvös Loránd Universität, Wissenschaftliche Fakultät, Mittelschullehrerin in Biologie 2001-
Diplomfach:	Evolutionsbiologie, Ökologie und Systematik
Diplomarbeit:	„Diasporenbank der Kryptogamen in Dolomitfelsrasen“. Lehrstuhl für Pflanzensystematik und Ökologie, Eötvös Loránd Universität, Wissenschaftliche Fakultät, Budapest, Ungarn Dr. Zoltán Tóth. 2003
Doktorat:	Universität Zürich, Institut für Systematische Botanik, Dr. Edwin Urmi und Dr. Jakob Schneller.

Publikationsliste:

Hock Zs, Szövényi P, Tóth Z 2004 Seasonal variation in the diaspore bank of bryophytes in open dolomite rock grasslands. *Journal of Bryology* 26: 285-292

Szövényi P, Hock Zs, Tóth Z 2004 Phorophyte preferences of epiphytic bryophytes in a stream valley in the Carpathian Basin. *Journal of Bryology* 26: 137-146

Szövényi P, Hock Zs, Urmi E, Schneller JJ 2006 New primers for amplifying the GapC gene of bryophytes and its utility in intraspecific phylogenies in the genus *Sphagnum*. *Lindbergia* 31: 78-84

Szövényi P, Hock Zs, Urmi E, Schneller JJ 2006 Contrasting phylogeographic patterns in *Sphagnum fimbriatum* and *S. squarrosum* (Bryophyta, Sphagnopsida) in Europe. *New Phytologist* 172: 784-794

Hock Zs, Szövényi P, Tóth Z 2006 Seasonal variation in the spore bank of ferns in grasslands on dolomite rock. *Plant Ecology* 187: 289-296

Articles in review

Szövényi P, Hock Zs, Schneller JJ 2006 Does multilocus dataset support cryptic demographic histories in two peat mosses in Europe? In review, *BMC Evolutionary Biology*

Hock Zs, Szövényi P, Tóth Z 2007 Effects of habitat type and seasons on the composition of the bryophyte diaspore bank and its relation to the standing vegetation. In review: *Plant Ecology*

Hock Zs, Szövényi P, Schneller JJ, Urmi E, Tóth Z 2007 Population genetic consequences of the reproductive system in the liverwort *Mannia fragrans* (Aytoniaceae) In review: *American Journal of Botany*